

FORM-PCT-1390
(Rev. 12-29-99)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

030560-057

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)

To be assigned **09/913858**INTERNATIONAL APPLICATION NO.
PCT/AT00/00040INTERNATIONAL FILING DATE
17 February 2000PRIORITY DATE CLAIMED
18 February 1999

TITLE OF INVENTION

FUCOSYL TRANSFERASE GENE

APPLICANT(S) FOR DO/EO/US

Friedrich ALTMANN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
 - ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

PCT Notification Concerning Submission or Transmittal of Priority Document
 PCT Notice Informing the Applicant of the Communication of the International Application to the Designated Offices
 International Preliminary Examination Report
 Cover page of published PCT international application (WO 00/49153)
 PCT Request
 Replacement Sheet of claims

U.S. APPLICATION NO. (if known, see 37 CFR 1.55) **09/913858** INTERNATIONAL APPLICATION NO. **PCT/AT00/00040** ATTORNEY'S DOCKET NUMBER **030560-057**
 To be assigned

17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS		PTO USE ONLY	
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,000.00 (960) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 (970) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 (958) International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 (956) International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962)							
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$	860.00		
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).				20	<input type="checkbox"/>	30	<input type="checkbox"/>
				\$			
Claims		Number Filed	Number Extra	Rate			
Total Claims		73 -20 =	53	X\$18.00 (966)		\$	954.00
Independent Claims		15 -3 =	12	X\$80.00 (964)		\$	960.00
Multiple dependent claim(s) (if applicable)				+ \$270.00 (968)		\$	
TOTAL OF ABOVE CALCULATIONS =				\$	2,774.00		
Reduction for 1/2 for filing by small entity, if applicable (see below).				\$	1,387.00		
SUBTOTAL =				\$			
Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)).				20	<input type="checkbox"/>	30	<input type="checkbox"/>
				\$			
TOTAL NATIONAL FEE =				\$	1,387.00		
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$			
TOTAL FEES ENCLOSED =				\$	1,387.00		
				Amount to be refunded		\$	
				charged		\$	
a. <input checked="" type="checkbox"/> Small entity status is hereby claimed. b. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1,387.00</u> to cover the above fees is enclosed. c. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. d. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed. NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.							
SEND ALL CORRESPONDENCE TO:							
Ronald L. Grudziecki BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620							
				SIGNATURE <u><i>Donna M. Meuth</i></u> NAME <u>Donna M. Meuth</u>			
				36,607 REGISTRATION NUMBER			

Patent
Attorney's Docket No. 030560-057

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Friedrich ALTMANN)	Group Art Unit: To be assigned
)	
Application No.: U.S. National Phase of)	Examiner: To be assigned
PCT/AT00/00040, filed February 17, 2000)	
)	
Filed: August 20, 2001)	
)	
For: FUCOSYL TRANSFERASE GENE)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments.

IN THE CLAIMS:

Please delete the current claims 1-34 without prejudice or disclaimer.

Please insert new claims 35-107 as follows:

--35. A DNA molecule comprising a sequence selected from the group consisting of

(A) a sequence according to SEQ ID NO 1 with an open reading frame from base pair 211 to base pair 1740,

(B) a sequence which is at least 50% homologous with the sequence according to

SEQ ID NO 1, and

(C) a sequence which hybridizes with the sequence according to SEQ ID NO 1 under stringent conditions,

wherein said sequence (A), (B) and (C) codes for a plant protein having fucosyl transferase activity.

36. A DNA molecule comprising a sequence complementary to a sequence selected from the group consisting of

(A) a sequence according to SEQ ID NO 1 with an open reading frame from base pair 211 to base pair 1740,

(B) a sequence which is at least 50% homologous with the sequence according to SEQ ID NO 1, and

(C) a sequence which hybridizes with the sequence according to SEQ ID NO 1 under stringent conditions,

wherein said sequence of (A), (B) and (C) codes for a plant protein having fucosyl transferase activity.

37. The DNA molecule according to claim 35 coding for a protein having GlcNAc- α 1,3-fucosyl transferase activity.

38. The DNA molecule according to claim 35 coding for a protein having core- α 1,3-fucosyl transferase activity.

39. The DNA molecule according to claim 35, wherein said sequence is at least 70-80% homologous with the sequence according to SEQ ID NO 1.

40. The DNA molecule according to claim 35, wherein said sequence is at least 95% homologous with the sequence according to SEQ ID NO 1.

41. The DNA molecule according to claim 35 comprising between about 2150 and about 2250 base pairs.

42. The DNA molecule according to claim 35 comprising about 2198 base pairs.

43. A DNA molecule comprising a sequence selected from the group consisting of

a sequence according to SEQ ID NO 3,

a sequence which is at least 85% homologous with the sequence according to SEQ ID NO 3, and

a sequence which hybridizes with the sequence according to SEQ ID NO 3 under stringent conditions.

44. A DNA according to claim 43, wherein the sequence is at least 95% homologous with the sequence according to SEQ ID NO 3.

45. A DNA molecule comprising a partial sequence of said DNA molecule according to claim 35, wherein said sequence is at least 80% homologous with said SEQ ID NO 1 and has a size of 20 to 200 base pairs.

46. The DNA molecule according to claim 45, said molecule being covalently associated with a detectable marker substance.

47. A biologically functional vector comprising said DNA molecule according to claim 35.

48. A biological vector comprising parts of said DNA molecule according to claim 35 of different length, said parts having at least 20 base pairs.

49. A biologically functional vector comprising said DNA molecule according to claim 35, wherein said DNA molecule is inversely orientated with respect to the promotor.

50. A biologically functional vector comprising parts of said DNA molecule according to claim 35 of different lengths, wherein said DNA molecule parts are inversely orientated with respect to the promotor.

51. A DNA molecule coding for a ribozyme having two sequence sections, wherein each sequence section has a length of at least 10 to 15 base pairs, and

is complementary to a sequence section of said DNA molecule according to claim 35,

wherein said ribozyme complexes and cuts the mRNA transcribed by a natural GlcNAc- α 1,3-fucosyl transferase DNA molecule.

52. A biologically functional vector comprising said DNA molecule according to claim 51.

53. A method of preparing a cDNA comprising a DNA molecule according to claim 35 comprising

isolating RNA from cells selected from the group consisting of insect cells, plant cells and hypocotylous cells, and

adding a reverse transcriptase and primers thereto to effect a reverse transcription with said RNA and prepare said cDNA.

54. A method of cloning a GlcNAc- α 1,3-fucosyl transferase comprising
cloning said DNA molecule according to claim 35 into a vector,
transfecting said vector into a host,
selecting and amplifying transfected host cells, wherein said host cell lines express the active GlcNAc- α 1,3-fucosyl transferase.

55. A method of preparing recombinant hosts selected from the group consisting

of host cells, plant cells, insect cells, plants and insects, wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or inhibited, comprising

inserting into a recombinant host, a biologically functional vector which comprises a DNA molecule according to claim 35, wherein said DNA sequence comprises a deletion, insertion or substitution mutation.

56. A method of preparing recombinant hosts selected from the group consisting of host cells, plant cells, insect cells, plants and insects wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or inhibited, comprising

inserting into a recombinant host, a biologically functional vector which comprises parts of said DNA molecule according to claim 35 of different length, said parts having at least 20 base pairs, wherein said DNA sequence comprises a deletion, insertion or substitution mutation.

57. A method of preparing recombinant hosts selected from the group consisting of host cells, plant cells, insect cells, plants and insects wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or inhibited, comprising

inserting into a recombinant host, a biologically functional vector which comprises a DNA molecule according to claim 35, wherein said DNA sequence is inversely orientated with respect to the promoter.

58. A method of preparing recombinant hosts selected from the group consisting

of host cells, plant cells, insect cells, plants and insects wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or inhibited, comprising

inserting into a recombinant host, a biologically functional vector which comprises parts of said DNA molecule according to claim 35 of different length, said parts having at least 20 base pairs, wherein said DNA sequence is inversely orientated with respect to the promoter.

59. A method of preparing recombinant hosts selected from the group consisting of host cells, plant cells, insect cells, plants and insects wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or inhibited, comprising

inserting into a recombinant host, a biologically functional vector which comprises a DNA molecule according to claim 51, wherein said DNA sequence is inversely orientated with respect to the promoter.

60. A method of preparing recombinant hosts selected from the group consisting of host cells, plant cells, insect cells, plants and insects wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or inhibited, comprising

inserting into a recombinant host, a biologically functional vector which comprises a DNA molecule according to claim 51, wherein said DNA sequence comprises a deletion, insertion or substitution mutation.

61. A method of preparing recombinant hosts selected from the group consisting

of host cells, plant cells, insect cells, plants and insects, comprising

inserting a DNA molecule according to claim 35 into the genome of said host at the position of a non-mutated, homologous sequence,

wherein said DNA sequence comprises a deletion, insertion or substitution mutation.

62. A recombinant host prepared according to said method according to claim 60, wherein its GlcNAc- α 1,3-fucosyl transferase production is suppressed.

63. A recombinant host prepared according to said method according to claim 60, wherein its GlcNAc- α 1,3-fucosyl transferase production is completely inhibited.

64. A recombinant host prepared according to said method according to claim 61, wherein its GlcNAc- α 1,3-fucosyl transferase production is suppressed.

65. A recombinant host prepared according to said method according to claim 61, wherein its GlcNAc- α 1,3-fucosyl transferase production is completely inhibited.

66. A peptide nucleic acid molecule comprising a base sequence complementary to the sequence of said DNA molecule according to claim 35.

67. A peptide nucleic acid molecule comprising a partial sequence of a base

sequence complementary to the sequence of said DNA molecule according to claim 35.

68. A peptide nucleic acid molecule comprising a base sequence corresponding to the sequence of said DNA molecule according to claim 35.

69. A peptide nucleic acid molecule comprising a partial sequence of the sequence of said DNA molecule according to claim 35.

70. A method of producing a host selected from the group consisting of plants, insects, cells, plant cells and insect cells having blocked expression of GlcNAc- α 1,3-fucosyl transferase, comprising
inserting into said host a peptide nucleic acid molecule according to claim 66.

71. The method according to claim 70, wherein said expression is blocked at the transcription or translation level.

72. A method of producing a host selected from the group consisting of plants, insects, cells, plant cells and insect cells having blocked expression of GlcNAc- α 1,3-fucosyl transferase, comprising
inserting into said host a peptide nucleic acid molecule according to claim 66.

73. The method according to claim 72, wherein said expression is blocked at the transcription or translation level.

74. A method of producing a host selected from the group consisting of plants, insects, cells, plant cells and insect cells having blocked expression of GlcNAc- α 1,3-fucosyl transferase, comprising

inserting into said host a peptide nucleic acid molecule according to claim 68.

75. The method according to claim 74, wherein said expression is blocked at the transcription or translation level.

76. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 62, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

77. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 63, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

78. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 64, with a gene that expresses
said glycoprotein, and
expressing said recombinant glycoprotein.

79. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 65, with a gene that expresses
said glycoprotein, and
expressing said recombinant glycoprotein.

80. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host, which is prepared according to the method of claim
70, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

81. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host, which is prepared according to the method of claim
72, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

82. A method of producing recombinant glycoprotein, comprising
transfecting a recombinant host, which is prepared according to the method of claim

74, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

83. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 62, with a gene that expresses
said glycoprotein, and
expressing said recombinant glycoprotein.

84. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 63, with a gene that expresses
said glycoprotein, and
expressing said recombinant glycoprotein.

85. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 64, with a gene that expresses
said glycoprotein, and
expressing said recombinant glycoprotein.

86. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host according to claim 65, with a gene that expresses
said glycoprotein, and
expressing said recombinant glycoprotein.

87. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host, which is prepared according to the method of claim
70, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

88. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host, which is prepared according to the method of claim
72, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

89. A method of producing human recombinant glycoprotein, comprising
transfecting a recombinant host, which is prepared according to the method of claim
74, with a gene that expresses said glycoprotein, and
expressing said recombinant glycoprotein.

90. A method of selecting DNA molecules coding for a GlcNAc- α 1,3-fucosyl
transferase comprising

(A) adding to a sample DNA molecules according to claim 46, and

(B) selecting molecules which bind to said DNA molecules of (A), wherein the
molecules which bind code for a GlcNAc- α 1,3-fucosyl transferase.

91. The method according to claim 90, wherein said sample comprises genomic DNA of a plant.

92. The method according to claim 90, wherein said sample comprises genomic DNA of an insect organism.

93. DNA molecules coding for a GlcNAc- α 1,3-fucosyl transferase selected according to a method according to claim 90, and isolated from said sample.

94. DNA molecules coding for a GlcNAc- α 1,3-fucosyl transferase selected according to a method according to claim 91, and isolated from said sample.

95. DNA molecules coding for a GlcNAc- α 1,3-fucosyl transferase selected according to a method according to claim 92, and isolated from said sample.

96. A DNA molecule comprising a sequence according to SEQ ID NO 1 with an open reading frame from base pair 211 to base pair 1740.

97. A DNA molecule which is at least 70-80% homologous with the sequence according to SEQ ID NO 1, and codes for a plant protein having fucosyl transferase activity.

98. A DNA molecule which is at least 95% homologous with the sequence according to SEQ ID NO 1, and codes for a plant protein having fucosyl transferase activity.

99. A DNA molecule which hybridizes with the sequence according to SEQ ID NO 1 under stringent conditions, and codes for a plant protein having fucosyl transferase activity.

100. A DNA molecule comprising a sequence complementary to a sequence according to SEQ ID NO 1 with an open reading frame from base pair 211 to base pair 1740.

101. A DNA molecule comprising a sequence complementary to a sequence which (A) is at least 70-80% homologous with the sequence according to SEQ ID NO 1, and
and
(B) codes for a plant protein having fucosyl transferase activity.

102. A DNA molecule comprising a sequence complementary to a sequence which (A) is at least 95% homologous with the sequence according to SEQ ID NO 1, and
(B) codes for a plant protein having fucosyl transferase activity.

103. A DNA molecule comprising a sequence complementary to a sequence which
(A) hybridizes with the sequence according to SEQ ID NO 1 under stringent
conditions, and

(B) codes for a plant protein having fucosyl transferase activity.

104. A DNA molecule comprising a sequence according to SEQ ID NO 3.

105. A DNA molecule comprising a sequence which is at least 85% homologous
with a sequence according to SEQ ID NO 3.

106. A DNA molecule comprising a sequence which hybridizes with the sequence
according to SEQ ID NO 3 under stringent conditions.

107. A DNA molecule comprising a sequence which is at least 95% homologous
with a sequence according to SEQ ID NO 3.--

REMARKS

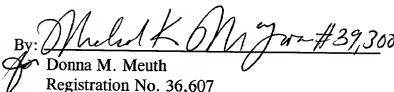
Entry of the foregoing and examination of the above-identified application are respectfully requested.

The original claims 1-34 have been deleted in favor of new claims 35-107. Support for these claims may be found in the original claims. No new matter has been added by these claims.

In the event that there are any questions relating to this Preliminary Amendment, or to the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at 508-339-3684 concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By:  #39,300
Donna M. Meuth
Registration No. 36,607

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: August 20, 2001



PCT

WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 7 : C12N 15/54, 9/10, 15/11, 9/00, 5/10, C12P 21/00, C12Q 1/68		A1	(11) Internationale Veröffentlichungsnummer: WO 00/49153 (43) Internationales Veröffentlichungsdatum: 24. August 2000 (24.08.00)
(21) Internationales Aktenzeichen: PCT/AT00/00040 (22) Internationales Anmeldedatum: 17. Februar 2000 (17.02.00) (30) Prioritätsdaten: A 270/99 18. Februar 1999 (18.02.99) AT (71)(72) Anmelder und Erfinder: ALTMANN, Friedrich [AT/AT]; Institut für Chemie, Universität für Bodenkultur, Muthgasse 18, A-1190 Wien (AT). (74) Anwälte: SONN, Helmut usw., Riemergasse 14, A-1010 Wien (AT).		(81) Bestimmungsstaaten: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO Patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Veröffentlicht <i>Mit internationalem Recherchenbericht. Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist; Veröffentlichung wird wiederholt falls Änderungen eintreffen.</i>	
(54) Title: FUCOSYL TRANSFERASE GENE			
(54) Bezeichnung: FUCOSYLTRANSFERASE-GEN			
(57) Abstract <p>The invention relates to a DNA molecule which comprises a sequence according to SEQ ID No: 1 with an open reading frame of base pair 211 to base pair 1740 or which exhibits at least 50 % homology to the above-mentioned sequence, or which is hybridized with the above-mentioned sequence under stringent conditions, or which comprises a sequence that is degenerated to the above-mentioned DNA sequence due to the genetic code. The sequence codes for a vegetable protein comprising a fucosyl transferase activity or is complementary thereto.</p>			
(57) Zusammenfassung <p>Es wird ein DNA-Molekül zur Verfügung gestellt, das eine Sequenz gemäß der SEQ ID No: 1 mit einem offenen Leserahmen von Basenpaar 211 bis Basenpaar 1740 umfasst oder zumindest 50 % Homologie zur obengenannten Sequenz aufweist oder unter stringenten Bedingungen mit der oben genannten Sequenz hybridisiert oder eine Sequenz umfasst, die infolge des genetischen Codes zur oben genannten DNA-Sequenz degeneriert ist, wobei die Sequenz für ein pflanzliches Protein mit einer Fucosyltransferase-Aktivität codiert oder dazu komplementär ist.</p>			

16/pst>

The invention relates to polynucleotides coding for a fucosyl transferase. Furthermore, the invention relates to partial sequences of these polynucleotides as well as to vectors comprising these polynucleotides, recombinant host cells, plants and insects transfected with the polynucleotides or with DNA derived therefrom, respectively, as well as to glycoproteins produced in these systems.

Glycoproteins exhibit a variety and complexity of carbohydrate units, the composition and arrangement of the carbohydrates being characteristic of different organisms. The oligosaccharide units of the glycoproteins have a number of tasks, e.g. they are important in regulating metabolism, they are involved in transmitting cell-cell interactions, they determine the circulation periods of proteins in circulation, and they are decisive for recognizing epitopes in antigen-antibody reactions.

The glycosylation of glycoproteins starts in the endoplasmic reticulum (ER), where the oligosaccharides are either bound to asparagine side chains by N-glycosidic bonds or to serine or threonine side chains by O-glycosidic bonds. The N-bound oligosaccharides contain a common core from a penta-saccharide unit which consists of three mannose and two N-acetyl glucose amine residues. To further modify the carbohydrate units, the proteins are transported from the ER to the Golgi complex. The structure of the N-bound oligosaccharide units of glycoproteins is determined by their conformation and by the composition of the glycosyl transferases of the Golgi compartments in which they are processed.

It has been shown that the core pentasaccharide unit in the Golgi complex of some plant and insect cells is substituted by xylose and α 1,3-bound fucose (P. Lerouge et al., 1998, Plant Mol. Biol. 38, 31-48; Rayon et al., 1998, L. Exp. Bot. 49, 1463-1472). The heptasaccharide "MMXF³" forming constitutes the main oligosaccharide type in plants (Kurosaka et al., 1991, J. Biol. Chem., 266, 4168-4172). Thus, e.g., the horseradish peroxidase, carrot β -fructosidase and Erythrina cristagalli comprise lectin as well as the honeybee venom phospholipase A2 or the neuronal membrane glycoproteins from insect embryos α 1,3-fucose residues which are bound to the glycan core. These structures are also termed complex N-glycans or mannose-deficient or truncated N-glycans, respectively. The α -mannosyl residues may be further replaced by

GlcNAc, to which galactose and fucose are bound so that a structure is prepared which corresponds to the human Lewis a-epitope (Melo et al., 1997, FEBS Lett 415, 186-191; Fitchette-Laine et al., 1997, Plant J. 12, 1411-1417).

Neither xylose nor the α 1,3-bound fucose exist in mammalian glycoproteins. It has been found that the core- α 1,3-fucose plays an important role in the epitope recognition of antibodies which are directed against plant and insect N-bound oligosaccharides (I.B.H. Wilson et al., Glycobiology Vol. 8, No. 7, pp. 651-661, 1998), and thereby trigger immune reactions in human or animal bodies against these oligosaccharides. The α 1,3-fucose residue furthermore seems to be one of the main causes for the wide-spread allergic cross reactivity between various plant and insect allergens (Tretter et al., Int. Arch. Allergy Immunol. 1993; 102:259-266) and is also termed "cross-reactive carbohydrate determinant" (CCD). In a study of epitopes of tomatoes and grass pollen, also α 1,3-bound fucose residues were found as a common determinant, which seems to be the reason why tomato and grass pollen allergies frequently occur together in patients (Petersen et al., 1996, J. Allergy Clin. Immunol., Vol. 98, 4; 805-814). Due to the frequent occurrence of immunological cross reactions, the CCDs moreover mask allergy diagnoses.

The immunological reactions triggered in the human body by plant proteins are the main problem in the medicinal use of recombinant human proteins produced in plants. To circumvent this problem, α 1,3-core-fucosylation would have to be prevented. In a study it could be demonstrated that oligosaccharides comprising an L-galactose instead of an L-fucose (6-deoxy-L-galactose) nevertheless are biologically fully active (E. Zablackis et al., 1996, Science, Vol. 272). According to another study, a mutant of the plant *Arabidopsis thaliana* was isolated in which the N-acetyl-glucosaminyl transferase I, the first enzyme in the biosynthesis of complex glycans, is missing. The biosynthesis of the complex glycoproteins in this mutant thus is disturbed. Nevertheless, these mutant plants are capable of developing normally under certain conditions (A. Schaewen et al, 1993, Plant Physiol. 102; 1109-1118).

To purposefully block the binding of the core- α 1,3-fucose in an oligosaccharide without also interfering in other glycosylation steps, merely that enzyme would have to be inactivated which

is directly responsible for this specific glycosylation, i.e. the core- α 1,3-fucosyl transferase. It has been isolated and characterized for the first time from mung beans, and it has been found that the activity of this enzyme depends on the presence of non-reducing GlcNAc ends (Staudacher et al., 1995, Glycoconjugate J. 12, 780-786). This transferase which only occurs in plants and insect, yet not in human beings or in other vertebrates, would have to be inactivated on purpose or suppressed so that human proteins which are produced in plants or in plant cells or also in insects or in insect cells, respectively, do no longer comprise this immune-reaction-triggering epitope, as has been the case so far.

The publication by John M. Burke "Clearing the way for ribozymes" (Nature Biotechnology 15:414-415; 1997) relates to the general mode of function of ribozymes.

The publication by Pooga et al., "Cell penetrating PNA constructs regulate galanin receptor levels and modify pain transmission in vivo" (Nature Biotechnology 16:857-861; 1998) relates to PNA molecules in general and specifically to a PNA molecule that is complementary to human galanin receptor type 1 mRNA.

US 5,272,066 A relates to a method of changing eukaryotic and prokaryotic proteins to prolongue their circulation in vivo. In this instance, the bound oligosaccharides are changed with the help of various enzymes, among them also GlcNAc- α 1 \rightarrow 3(4)-fucosyl transferase.

EP 0 643 132 A1 relates to the cloning of an α 1,3-fucosyl transferase isolated from human cells (THP-1). The carbohydrate chains described in this publication correspond to human sialyl Lewis x- and sialyl Lewis a-oligosaccharides. The specificity of the enzyme from human cells is quite different than that of fucosyltransferase from plant cells.

It is an object of the present invention to clone and to sequence the gene which codes for a plant fucosyl transferase, and to prepare vectors comprising this gene, DNA fragments thereof or an altered DNA or a DNA derived therefrom, to transfect plants and insects as well as cells thereof with one of these vectors, to produce glycoproteins that do not comprise the normally occurring α 1,3-core-fucose, as well as to provide corresponding methods therefor.

The object according to the invention is achieved by a DNA

molecule comprising a sequence according to SEQ ID NO: 1 (in this disclosure also the IUPAC code has been used, "N" meaning inosin) with an open reading frame from base pair 211 to base pair 1740 or being at least 50% homologous to the above sequence or hybridizing with the above-indicated sequence under stringent conditions, or comprising a sequence which has degenerated to the above DNA sequence due to the genetic code, the sequence coding for a plant protein which has fucosyl transferase activity or is complementary thereto.

This sequence which has not been described before can be perfectly used for any experiments, analysis and methods for production etc. which relate to the plant fucosyl transferase activity. Here the DNA sequence as well as the protein coded by this sequence are of interest. However, in particular the DNA sequence will be used for the inhibition of the fucosyl transferase activity.

The open reading frame of the SEQ ID NO: 1 codes for a protein with 510 amino acids and with a theoretical molecular weight of 56.8 kDa, a transmembrane portion presumably being present in the region between Asn36 and Gly54. The calculated pI value of the encoded protein of the sequence according to SEQ ID NO: 1 is 7.51.

The activity of the plant fucosyl transferase is detected by a method and measured, the fucosyl transferase being added to a sample comprising labelled fucose and an acceptor (e.g. a glycoprotein) bound to a carrier, e.g. Sepharose. After the reaction time, the sample is washed, and the content of bound fucose is measured. The activity of the fucosyl transferase in this case is seen as positive if the activity measurement is higher by at least 10 to 20%, in particular at least 30 to 50%, than the activity measurement of the negative control. The structure of the glycoprotein may additionally be verified by means of HPLC. Such protocols are prior art (Staudacher et al. 1998, Anal. Biochem. 246, 96-101; Staudacher et al. 1991, Eur. J. Biochem. 199, 745-751).

For example, fucosyl transferase is admixed to a sample comprising radioactively labelled fucose and an acceptor, e.g. GlcNAc β 1-2Man α 1-3(GlcNAc β 1-2Man α 1-6)Man β 1-4GlcNAc β 1-4GlcNAc β 1-Asn. After the reaction time, the sample is purified by anion exchange chromatography, and the content of bound fucose is measured.

From the difference of the measured radioactivity of the sample with acceptor and that of a negative control without acceptor, the activity can be calculated. The activity of the fucosyl transferase is already evaluated as positive if the radioactivity measured is at least 30-40% higher than the measured radioactivity of the negative sample.

The pairing of two DNA molecules can be changed by selection of the temperature and ionic strength of the sample. By stringent conditions, according to the invention conditions are understood which allow for an exact, stringent, binding. For instance, the DNA molecules are hybridized in 7% sodium dodecyl sulfate (SDS), 0.5M NaPO₄, pH 7.0, 1mM EDTA at 50°C, and washed with 1% SDS at 42°C.

Whether sequences have an at least 50% homology to SEQ ID NO: 1 can be determined e.g. by means of the program FastDB of EMBL or SWISSPROT data bank.

Preferably, the sequence of the DNA molecule of the invention encodes a protein with a GlcNAc- α 1,3-fucosyl transferase activity, in particular with a core- α 1,3-fucosyl transferase activity.

As described above the core of α 1,3-fucosyl transferase is present in plants and insects, however, not in the human body, so that in particular this DNA sequence is useful in analysis and experiments as well as methods for production which are fucosyl transferase specific.

By a core- α 1,3-fucosyl transferase, in particular GDP-L-Fuc:Asn-bound GlcNAc- α 1,3-fucosyl transferase is understood. Within the scope of the present invention, the term α 1,3-fucosyl transferase as a rule particularly means core- α 1,3 fucosyl transferase. For the above-described activity measurement, in particular acceptors having a non-reducing GlcNAc terminus are used. Such acceptors are, e.g., GlcNAc β 1-2Man α 1-3 (GlcNAc β 1-2Man α 1-6)Man β 1-4GlcNAc β 1-4GlcNAc β 1-Asn, GlcNAc β 1-2Man α 1-3 (GlcNAc β 1-2Man α 1-6)Man β 1-4GlcNAc β 1-4 (Fuc α 1-6)GlcNAc β 1-Asn and GlcNAc β 1-2Man α 1-3 [Man α 1-3 (Man α 1-6)Man α 1-6]Man β 1-4GlcNAc β 1-4GlcNAc β 1-Asn. Whether the fucose is bound or not can furthermore be determined by measuring the insensitivity relative to N-glycosidase F, which can be detected by means of mass spectrometry.

Preferably, the DNA molecule according to the invention comprises at least 70-80%, particularly preferred at least 95%, ho-

mology to the sequence according to SEQ ID NO: 1. This sequence codes for a particularly active GlcNAc- α 1,3-fucosyl transferase.

Since the DNA sequence can be more or less changed according to the plant or the insect a sequence which shows, for example, 70 % homology to a sequence according to SEQ ID No 1 has also a fucosyl transferase activity which is sufficient in order to be used in analysis, experiments or methods of production as above described.

According to a further advantageous embodiment, the DNA molecule comprises 2150 to 2250, in particular 2198, base pairs. This DNA molecule comprises 100 to 300, preferably 210, base pairs upstream in front of the start codon, as well as 350 to 440, in particular 458, base pairs downstream after the stop codon of the open reading frame, wherein the end of the DNA molecule preferably comprises a 3'-poly(A)-tail. In this manner, a faultless regulation on translation level is ensured and a DNA molecule is provided which is particularly efficient and unproblematic for the coding of an active GlcNAc- α 1,3-fucosyl transferase.

The present invention moreover relates to a DNA molecule which comprises a sequence according to SEQ ID NO: 3 or comprising a sequence having at least 85%, particularly preferred at least 95%, in particular at least 99%, homology to the above-identified sequence or which, under stringent conditions, hybridizes with the above-indicated sequence or which has degenerated to the above-indicated DNA sequence due to the genetic code. The homology preferably is determined with a program which recognizes insertions and deletions and which does not consider these in the homology calculation. This nucleotide sequence codes for a conserved peptide motif, which means that the plurality of the active and functioning GlcNAc- α 1,3-fucosyl transferases comprises the amino acid sequence encoded thereby. In this instance, the sequence may either have the same size as the sequence according to SEQ ID NO: 3, or, of course, it may also be larger. This sequence has a smaller length than the sequence which codes the complete protein and is therefore less sensitive with respect to recombination, deletion, or any other mutations. Due to the conservative motif and its higher stability this sequence is particularly advantageous for sequence recognising test.

SEQ ID NO: 3 comprises the following sequence:

5'-GAAGCCCTGAAGCACTACAAATTAGCTTAGCGTTTGAAAATTCGAATGAGGAAG
ATTATGTAAGTGA AAAATCTCTCCAATCCCTTGTGCTGGAAGTGCCT-3'

In a further aspect, the present invention relates to a DNA molecule which comprises a partial sequence of one of the above-indicated DNA molecules and has a size of from 20 to 200, preferably from 30 to 50, base pairs. The DNA molecule may, e.g., be utilized to bind, as a probe, to complementary sequences of GlcNAc- α 1,3-fucosyl transferases so that they can be selected from a sample. In this manner, further GlcNAc- α 1,3-fucosyl transferases from the most varying plants and insects can be selected, isolated and characterized. Any desired one or also several different partial sequences may be used, in particular a part of the conserved motif already described above.

In doing so, it is particularly advantageous if one of the above-indicated DNA molecules is covalently associated with a detectable labelling substance. As the labelling substance, any common marker can be used, such as, e.g., fluorescent, luminescent, radioactive markers, non-isotopic markers, such as biotin, etc. In this manner, reagents are provided which are suitable for the detection, selection and quantitation of corresponding DNA molecules in solid tissue samples (e.g. from plants) or also in liquid samples, by means of hybridizing methods.

A further aspect of the invention relates to a biologically functional vector which comprises one of the above-indicated DNA molecules or parts thereof of differing lengths with at least 20 base pairs. For transfection into host cells, an independent vector capable of amplification is necessary, wherein, depending on the host cell, transfection mechanism, task and size of the DNA molecule, a suitable vector can be used. Since a large number of different vectors is known, an enumeration thereof would go beyond the limits of the present application and therefore is done without here, particularly since the vectors are very well known to the skilled artisan (as regards the vectors as well as all the techniques and terms used in this specification which are known to the skilled artisan, cf. also Sambrook Maniatis). Ideally, the vector has a small molecule mass and should comprise selectable genes so as to lead to an easily recognizable phenotype in a cell so thus enable an easy selection of vector-containing and vector-free host cells. To obtain a high yield of DNA and corresponding gene products, the vector should comprise a

strong promoter, as well as an enhancer, gene amplification signals and regulator sequences. For an autonomous replication of the vector, furthermore, a replication origin is important. Polyadenylation sites are responsible for correct processing of the mRNA and splice signals for the RNA transcripts. If phages, viruses or virus particles are used as the vectors, packaging signals will control the packaging of the vector DNA. For instance, for transcription in plants, Ti plasmids are suitable, and for transcription in insect cells, baculoviruses, and in insects, respectively, transposons, such as the P element.

If the above-described inventive vector is inserted into a plant or into a plant cell, a post-transcriptional suppression of the gene expression of the endogenous $\alpha 1,3$ -fucosyl transferase gene is attained by transcription of a transgene homologous thereto or of parts thereof, in sense orientation. For this sense technique, furthermore, reference is made to the publications by Baucombe 1996, Plant. Mol. Biol., 9:373-382, and Brigneti et al., 1998, EMBO J. 17:6739-6746. This strategy of "gene silencing" is an effective way of suppressing the expression of the $\alpha 1,3$ -fucosyl transferase gene, cf. also Waterhouse et al., 1998, Proc. Natl. Acad. Sci. USA, 95:13959-13964.

Furthermore, the invention relates to a biologically functional vector comprising a DNA molecule according to one of the above-described embodiments, or parts thereof of differing lengths in reverse orientation to the promoter. If this vector is transfected in a host cell, an "antisense mRNA" will be read which is complementary to the mRNA of the GlcNAc- $\alpha 1,3$ -fucosyl transferase and complexes the latter. This bond will either hinder correct processing, transportation, stability or, by preventing ribosome annealing, it will hinder translation and thus the normal gene expression of the GlcNAc- $\alpha 1,3$ -fucosyl transferase.

Although the entire sequence of the DNA molecule could be inserted into the vector, partial sequences thereof because of their smaller size may be advantageous for certain purposes. With the antisense aspect, e.g., it is important that the DNA molecule is large enough to form a sufficiently large antisense mRNA which will bind to the transferase mRNA. A suitable antisense RNA molecule comprises, e.g., from 50 to 200 nucleotides since many of the known, naturally occurring antisense RNA molecules comprise approximately 100 nucleotides.

For a particularly effective inhibition of the expression of an active $\alpha 1,3$ -fucosyl transferase, a combination of the sense technique and the antisense technique is suitable (Waterhouse et al., 1998, Proc. Natl. Acad. Sci., USA, 95:13959-13964).

Advantageously, rapidly hybridizing RNA molecules are used. The efficiency of antisense RNA molecules which have a size of more than 50 nucleotides will depend on the annealing kinetics in vitro. Thus, e.g., rapidly annealing antisense RNA molecules exhibit a greater inhibition of protein expression than slowly hybridizing RNA molecules (Wagner et al., 1994, Annu. Rev. Microbiol., 48:713-742; Rittner et al., 1993, Nucl. Acids Res., 21:1381-1387). Such rapidly hybridizing antisense RNA molecules particularly comprise a large number of external bases (free ends and connecting sequences), a large number of structural subdomains (components) as well as a low degree of loops (Patzel et al. 1998; Nature Biotechnology, 16; 64-68). The hypothetical secondary structures of the antisense RNA molecule may, e.g., be determined by aid of a computer program, according to which a suitable antisense RNA DNA sequence is chosen.

Different sequence regions of the DNA molecule may be inserted into the vector. One possibility consists, e.g., in inserting into the vector only that part which is responsible for ribosome annealing. Blocking in this region of the mRNA will suffice to stop the entire translation. A particularly high efficiency of the antisense molecules also results for the 5'- and 3'-nontranslated regions of the gene.

Preferably, the DNA molecule according to the invention includes a sequence which comprises a deletion, insertion and/or substitution mutation. The number of mutant nucleotides is variable and varies from a single one to several deleted, inserted or substituted nucleotides. It is also possible that the reading frame is shifted by the mutation. In such a "knock-out gene" it is merely important that the expression of a GlcNAc- $\alpha 1,3$ -fucosyl transferase is disturbed, and the formation of an active, functional enzyme is prevented. In doing so, the site of the mutation is variable, as long as expression of an enzymatically active protein is prevented. Preferably, the mutation in the catalytic region of the enzyme which is located in the C-terminal region. The method of inserting mutations in DNA sequences are well known to the skilled artisan, and therefore the various possibilities

of mutageneses need not be discussed here in detail. Coincidental mutageneses as well as, in particular, directed mutageneses, e.g. the site-directed mutagenesis, oligonucleotide-controlled mutagenesis or mutageneses by aid of restriction enzymes may be employed in this instance.

The invention further provides a DNA molecule which codes for a ribozyme which comprises two sequence portions of at least 10 to 15 base pairs each, which are complementary to sequence portions of an inventive DNA molecule as described above so that the ribozyme complexes and cleaves the mRNA which is transcribed from a natural GlcNAc- α 1,3-fucosyl transferase DNA molecule. The ribozyme will recognize the mRNA of the GlcNAc- α 1,3-fucosyl transferase by complementary base pairing with the mRNA. Subsequently, the ribozyme will cleave and destroy the RNA in a sequence-specific manner, before the enzyme is translated. After dissociation from the cleaved substrate, the ribozyme will repeatedly hybridize with RNA molecules and act as specific endonuclease. In general, ribozymes may specifically be produced for inactivation of a certain mRNA, even if not the entire DNA sequence which codes for the protein is known. Ribozymes are particularly efficient if the ribosomes move slowly along the mRNA. In that case it is easier for the ribozyme to find a ribosome-free site on the mRNA. For this reason, slow ribosome mutants are also suitable as a system for ribozymes (J. Burke, 1997, Nature Biotechnology; 15, 414-415). This DNA molecule is particularly advantageous for the downregulation and inhibition, respectively, of the expression of plant GlcNAc- α 1,3-fucosyl transferases.

One possible way is also to use a varied form of a ribozyme, i.e. a minizyme. Minizymes are efficient particularly for cleaving larger mRNA molecules. A minizyme is a hammer head ribozyme which has a short oligonucleotide linker instead of the stem/loop II. Dimer-minizymes are particularly efficient (Kuwabara et al., 1998, Nature Biotechnology, 16; 961-965). Consequently, the invention also relates to a biologically functional vector which comprises one of the two last-mentioned DNA molecules (mutation or ribozyme-DNA molecule). What has been said above regarding vectors also applies in this instance. Such a vector can be, for example, inserted into a microorganism and can be used for the production of high concentrations of the above described DNA molecules. Furthermore such a vector is particu-

larly good for the insertion of a specific DNA molecule into a plant or an insect organism in order to downregulate or completely inhibit the GlcNAc- α 1,3-fucosyl transferase production in this organism.

According to the invention, there is provided a method of preparing a cDNA comprising the DNA molecule of the invention, wherein RNA is isolated from an insect or plant cell, in particular from hypocotyl cells, by means of which a reverse transcription is carried out after having admixed a reverse transcriptase and primers. The individual steps of this method are carried out according to protocols known per se. For the reverse transcription, on the one hand, it is possible to produce the cDNA of the entire mRNA with the help of oligo(dT) primers, and only then to carry out a PCR by means of selected primers so as to prepare DNA molecules comprising the GlcNAc- α 1,3-fucosyl transferase gene. On the other hand, the selected primers may directly be used for the reverse transcription so as to obtain short, specific cDNA. The suitable primers may be prepared e.g. synthetically according to the pattern of cDNA sequences of the transferase. With the help of this method big quantities of the inventive cDNA molecules can be produced quickly in a simple way and with few mistakes.

The invention furthermore relates to a method of cloning a GlcNAc- α 1,3-fucosyl transferase, characterized in that the DNA molecule of the invention is cloned into a vector which subsequently is transfected into a host cell or host, respectively, wherein, by selection and amplification of transfected host cells, cell lines are obtained which express the active GlcNAc- α 1,3-fucosyl transferase. The DNA molecule is inserted into the vector by aid of restriction endonucleases, e.g.. For the vector, there applies what has already been said above. What is important in this method is that an efficient host-vector system is chosen. To obtain an active enzyme, eukaryotic host cells are particularly suitable. One possible way is to transfect the vector in insect cells. In doing so, in particular an insect virus would have to be used as vector, such as, e.g., baculovirus.

Of course, human or other vertebrate cells can also be transfected, in which case the latter would express an enzyme foreign to them.

Preferably, a method of preparing recombinant host cells, in

particular plant or insect cells, or plants or insects, respectively, with a suppressed or completely stopped GlcNAc- α 1,3-fucosyl transferase production is provided, which is characterized in that at least one of the vectors according to the invention, i.e. that one comprising the inventive DNA molecule, the mutant DNA molecule or the DNA molecule coding for ribozymes or the one comprising the DNA molecule in inverse orientation to the promoter, is inserted into the host cell or plant or into the insect. What has been said above for the transfection also is applicable in this case.

As the host cells, plant cells may, e.g., be used, wherein, e.g., the Ti plasmid with the agrobacterium system is eligible. With the agrobacterium system it is possible to transfect a plant directly: agrobacteria cause root stem galls in plants. If agrobacteria infect an injured plant, the bacteria themselves do not get into the plant, but they insert the recombinant DNA portion, the so-called T-DNA, from the annular, extra chromosomal, tumour-inducing Ti-plasmid into the plant cells. The T-DNA, and thus also the DNA molecule inserted therein, are installed in the chromosomal DNA of the cell in a stable manner so that the genes of the T-DNA will be expressed in the plant.

There exist numerous known, efficient transfection mechanisms for different host systems. Some examples are electroporation, the calcium phosphate method, microinjection, liposome method.

Subsequently, the transfected cells are selected, e.g. on the basis of antibiotic resistences for which the vector comprises genes, or other marker genes. Then the transfected cell lines are amplified, either in small amounts, e.g. in Petri dishes, or in large amounts, e.g. in fermentors. Furthermore, plants have a particular characteristic, i.e. they are capable to re-develop from one (transfected) cell or from a protoplast, respectively, to a complete plant which can be grown.

Depending on the vector used, processes will occur in the host so that the enzyme expression will be suppressed or completely blocked:

If the vector comprising the DNA molecule with the deletion, insertion or substitution mutation is transfected, a homologous recombination will occur: the mutant DNA molecule will recognize the identical sequence in the genome of the host cell despite its

mutation and will be inserted exactly on that place so that a "knock-out gene" is formed. In this manner, a mutation is introduced into the gene for the GlcNAc- α 1,3-fucosyl transferase which is capable of inhibiting the faultless expression of the GlcNAc- α 1,3-fucosyl transferase. As has been explained above, with this technique it is important that the mutation suffices to block the expression of the active protein. After selection and amplification, the gene may be sequenced as an additional check so as to determine the success of the homologous recombination or the degree of mutation, respectively.

If the vector comprising the DNA molecule coding for a ribozyme is transfected, the active ribozyme will be expressed in the host cell. The ribozyme complexes the complementary mRNA sequence of the GlcNAc- α 1,3-fucosyl transferase at least at a certain site, cleaves this site, and in this manner it can inhibit the translation of the enzyme. In this host cell as well as in cell lines, or optionally, plant, respectively, derived therefrom, GlcNAc- α 1,3-fucosyl transferase will not be expressed. In case the vector comprises the inventive DNA molecule in sense or inverse direction to the promoter, a sense or antisense-mRNA will be expressed in the transfected cell (or plant, respectively). The antisense mRNA is complementary at least to a part of the mRNA sequence of the GlcNAc- α 1,3-fucosyl transferase and may likewise inhibit translation of the enzyme. As an example of a method of suppressing the expression of a gene by antisense technique, reference is made to the publication by Smith et al., 1990, Mol. Gen. Genet. 224:477-481, wherein in this publication the expression of a gene involved in the maturing process of tomatoes is inhibited.

In all the systems, expression of the GlcNAc- α 1,3-fucosyl transferase is at least suppressed, preferably even completely blocked. The degree of the disturbance of the gene expression will depend on the degree of complexing, homologous recombination, on possible subsequent coincidental mutations and on other processes in the region of the genome. The transfected cells are checked for GlcNAc- α 1,3-fucosyl transferase activity and selected.

Moreover, it is possible to still further increase the above-described suppression of the expression of the α 1,3-fucosyl transferase by introducing into the host a vector comprising a

gene coding for a mammalian protein, e.g. β 1,4-galactosyl transferase, in addition to the insertion of an above-described vector. Fucosylation may be reduced by the action of other mammalian enzymes, the combination of the inhibition of the expression of an active α 1,3-fucosyl transferase by means of the inventive vector and by means of a mammalian enzyme vector being particularly efficient.

Any type of plant may be used for transfection, e.g. mung bean, tobacco plant, tomato and/or potato plant. Another advantageous method of producing recombinant host cells, in particular plant or insect cells, or plants or insects, respectively, consists in that the DNA molecule comprising the mutation is inserted into the genome of the host cell, or plant or insect, respectively, in the place of the non-mutant homologous sequence (Schaefer et al., 1997, Plant J.; 11(6):1195-1206). This method thus does not function with a vector, but with a pure DNA molecule. The DNA molecule is inserted into the host e.g. by gene bombardment, microinjection or electroporation, to mention just three examples. As has already been explained, the DNA molecule binds to the homologous sequence in the genome of the host so that a homologous recombination and thus reception of the deletion, insertion or substitution mutation, respectively, will result in the genome: Expression of the GlcNAc- α 1,3-fucosyl transferase can be suppressed or completely blocked, respectively.

A further aspect of the invention relates to plants or plant cells, respectively, as well as insect or insect cells, respectively, their GlcNAc- α 1,3-fucosyl transferase activity being less than 50%, in particular less than 20%, particularly preferred 0%, of the GlcNAc- α 1,3-fucosyl transferase activity occurring in natural plants or plant cells, respectively, and insects or insect cells, respectively. The advantage of these plants or plant cells, respectively, is that the glycoproteins produced by them do not comprise any or hardly comprise any α 1,3-bound fucose. If products of these plants or insects, respectively, are taken up by human or vertebrate bodies, there will be no immune reaction to the α 1,3-fucose epitope.

Preferably, recombinant plants or plant cells, respectively, are provided which have been prepared by one of the methods described above, their GlcNAc- α 1,3-fucosyl transferase production

being suppressed or completely blocked, respectively.

The invention also relates to recombinant insects or insect cells, respectively, which have been prepared by one of the methods described above and whose GlcNAc- α 1,3-fucosyl transferase production is suppressed or completely blocked, respectively. Also in this instance, no glycoproteins having α 1,3-bound fucose residues are produced so that likewise no immune reaction to the α 1,3-fucose epitope will occur.

The invention also relates to a PNA molecule comprising a base sequence complementary to the sequence of the DNA molecule according to the invention as well as partial sequences thereof. PNA (peptide nucleic acid) is a DNA-like sequence, the nucleobases being bound to a pseudo-peptide backbone. PNA generally hybridizes with complementary DNA-, RNA- or PNA-oligomers by Watson-Crick base pairing and helix formation. The peptide backbone ensures a greater resistance to enzymatic degradation. The PNA molecule thus is an improved antisense agent. Neither nucleases nor proteases are capable of attacking a PNA molecule. The stability of the PNA molecule, if bound to a complementary sequence, comprises a sufficient steric blocking of DNA and RNA polymerases, reverse transcriptase, telomerase and ribosomes.

If the PNA molecule comprises the above-mentioned sequence, it will bind to the DNA or to a site of the DNA, respectively, which codes for GlcNAc- α 1,3-fucosyl transferase and in this way is capable of inhibiting transcription of this enzyme. As it is neither transcribed nor translated, the PNA molecule will be prepared synthetically, e.g. by aid of the the t-Boc technique. Advantageously, a PNA molecule is provided which comprises a base sequence which corresponds to the sequence of the inventive DNA molecule as well as partial sequences thereof. This PNA molecule will complex the mRNA or a site of the mRNA of GlcNAc- α 1,2-fucosyl transferase so that the translation of the enzyme will be inhibited. Similar arguments as set forth for the antisense RNA apply in this case. Thus, e.g., a particularly efficient complexing region is the translation start region or also the 5'-non-translated regions of mRNA.

A further aspect of the present invention relates to a method of preparing plants or insects, or cells, respectively, in particular plant or insect cells which comprise a blocked expression of the GlcNAc- α 1,3-fucosyl transferase on transcription or

translation level, respectively, which is characterized in that inventive PNA molecules are inserted in the cells. To insert the PNA molecule or the PNA molecules, respectively, in the cell, again conventional methods, such as, e.g., electroporation or microinjection, are used. Particularly efficient is insertion if the PNA oligomers are bound to cell penetration peptides, e.g. transportan or pAntp (Pooga et al., 1998, Nature Biotechnology, 16; 857-861).

The invention provides a method of preparing recombinant glycoproteins which is characterized in that the inventive, recombinant plants or plant cells, respectively, as well as recombinant insects or insect cells, respectively, whose GlcNAc- α 1,3-fucosyl transferase production is suppressed or completely blocked, respectively, or plants or insects, or cells, respectively, in which the PNA molecules have been inserted according to the method of the invention, are transfected with the gene that expresses the glycoprotein so that the recombinant glycoproteins are expressed. In doing so, as has already been described above, vectors comprising genes for the desired proteins are transfected into the host or host cells, respectively, as has also already been described above. The transfected plant or insect cells will express the desired proteins, and they have no or hardly any α 1,3-bound fucose. Thus, they do not trigger the immune reactions already mentioned above in the human or vertebrate body. Any proteins may be produced in these systems.

Advantageously, a method of preparing recombinant human glycoproteins is provided which is characterized in that the recombinant plants or plant cells, respectively, as well as recombinant insects or insect cells, respectively, whose GlcNAc- α 1,3-fucosyl transferase production is suppressed or completely blocked, or plants or insects, or cells, respectively, in which PNA molecules have been inserted according to the method of the invention, are transfected with the gene that expresses the glycoprotein so that the recombinant glycoproteins are expressed. By this method it becomes possible to produce human proteins in plants (plant cells) which, if taken up by the human body, do not trigger any immune reaction directed against α 1,3-bound fucose residues. There, it is possible to utilize plant types for producing the recombinant glycoproteins which serve as food stuffs, e.g. banana, potato and/or tomato. The tissues of this plant comprise

the recombinant glycoprotein so that, e.g. by extraction of the recombinant glycoprotein from the tissue and subsequent administration, or directly by eating the plant tissue, respectively, the recombinant glycoprotein is taken up in the human body. Preferably, a method of preparing recombinant human glycoproteins for medical use is provided, wherein the inventive, recombinant plants or plant cells, respectively, as well as recombinant insects or insect cells, respectively, whose GlcNAc- α 1,3-fucosyl transferase production is suppressed or completely blocked, respectively, or plants or insects, or cells, respectively, into which the PNA molecules have been inserted according to the method of the invention, are transfected with the gene that expresses the glycoprotein so that the recombinant glycoproteins are expressed. In doing so, any protein can be used which is of medical interest.

Moreover, the present invention relates to recombinant glycoproteins according to a method described above, wherein they have been prepared in plant or insect systems and wherein their peptide sequence comprises less than 50%, in particular less than 20%, particularly preferred 0%, of the α 1,3-bound fucose residues occurring in proteins expressed in non-fucosyl transferase-reduced plant or insect systems. Naturally, glycoproteins which do not comprise α 1,3-bound fucose residues are to be preferred. The amount of α 1,3-bound fucose will depend on the degree of the above-described suppression of the GlcNAc- α 1,3-fucosyl transferase.

Preferably, the invention relates to recombinant human glycoproteins which have been produced in plant or insect systems according to a method described above and whose peptide sequence comprises less than 50%, in particular less than 20%, particularly preferred 0%, of the α 1,3-bound fucose residues occurring in the proteins expressed in non-fucosyl transferase-reduced plant or insect systems.

A particularly preferred embodiment relates to recombinant human glycoproteins for medical use which have been prepared in plant or insect systems according to a method described above and whose peptide sequence comprises less than 50%, in particular less than 20%, particularly preferred 0%, of the α 1,3-bound fucose residues occurring in the proteins expressed in non-fucosyl transferase-reduced plant or insect systems.

The glycoproteins according to the invention may include other bound oligosaccharide units specific for plants or insects, respectively, whereby - in the case of human glycoproteins - they differ from these natural glycoproteins. Nevertheless, by the glycoproteins according to the invention, a slighter immune reaction or no immune reaction at all, respectively, is triggered in the human body, since, as has already been explained in the introductory portion of the specification, the $\alpha 1,3$ -bound fucose residues are the main cause for the immune reactions or cross immune reaction, respectively, to plant and insect glycoproteins.

A further aspect comprises a pharmaceutical composition comprising the glycoproteins according to the invention. In addition to the glycoproteins of the invention, the pharmaceutical composition comprises further additions common for such compositions. These are, e.g., suitable diluting agents of various buffer contents (e.g. Tris-HCl, acetate, phosphate, pH and ionic strength, additives, such as tensides and solubilizers (e.g. Tween 80, Polysorbate 80), preservatives (e.g. Thimerosal, benzyl alcohol), adjuvants, antioxidants (e.g. ascorbic acid, sodium metabisulfite), emulsifiers, fillers (e.g. lactose, mannitol), covalent bonds of polymers, such as polyethylene glycol, to the protein, incorporation of the material in particulate compositions of polymeric compounds, such as polylactic acid, polyglycolic acid, etc. or in liposomes, auxiliary agents and/or carrier substances which are suitable in the respective treatment. Such compositions will influence the physical condition, stability, rate of in vivo liberation and rate of in vivo excretion of the glycoproteins of the invention.

The invention also provides a method of selecting DNA molecules which code for a GlcNAc- $\alpha 1,3$ -fucosyl transferase, in a sample, wherein the labelled DNA molecules of the invention are admixed to the sample, which bind to the DNA molecules that code for a GlcNAc- $\alpha 1,3$ -fucosyl transferase. The hybridized DNA molecules can be detected, quantitated and selected. For the sample to contain single strand DNA with which the labelled DNA molecules can hybridize, the sample is denatured, e.g. by heating. One possible way is to separate the DNA to be assayed, possibly after the addition of endonucleases, by gele electrophoresis on an agarose gel. After having been transferred to a membrane of nitrocellulose, the labelled DNA molecules according to the in-

vention are admixed which hybridize to the corresponding homologous DNA molecule ("Southern blotting").

Another possible way consists in finding homologous genes from other species by PCR-dependent methods using specific and/or degenerated primers, derived from the sequence of the DNA molecule according to the invention.

Preferably, the sample for the above-identified inventive method comprises genomic DNA of a plant or insect organism. By this method, a large number of plants and insects is assayed in a very rapid and efficient manner for the presence of the GlcNAc- α 1,3-fucosyl transferase gene. In this manner, it is respectively possible to select plants and insects which do not comprise this gene, or to suppress or completely block, respectively, the expression of the GlcNAc- α 1,3-fucosyl transferase in such plants and insects which comprise this gene, by an above-described method of the invention, so that subsequently they may be used for the transfection and production of (human) glycoproteins. The invention also relates to DNA molecules which code for a GlcNAc- α 1,3-fucosyl transferase which have been selected according to the two last-mentioned methods and subsequently have been isolated from the sample. These molecules can be used for further assays. They can be sequenced and in turn can be used as DNA probes for finding GlcNAc- α 1,3-fucosyl transferases. These - labelled - DNA molecules will function for organisms, which are related to the organisms from which they have been isolated, more efficiently as probes than the DNA molecules of the invention. A further aspect of the invention relates to a preparation of GlcNAc- α 1,3-fucosyl transferase cloned according to the invention which comprises isoforms having pI values of between 6.0 and 9.0, in particular between 6.8 and 8.2. The pI values of a protein is that pH value at which its net charge is zero and is dependent on the amino acid sequence, the glycosylation pattern as well as on the spatial structure of the protein. The GlcNAc- α 1,3-fucosyl transferase comprises at least 7 isoforms which have a pI value in this range. The reason for the various isoforms of the transferase are, e.g., different glycosylations as well as limited proteolysis. Tests have shown that mung bean seedlings of various plants have different relationships of the isozymes. The pI value of a protein can be determined by isoelectric focussing, which is known to the skilled artisan.

The main isoform of the enzyme has an apparent molecular weight of 54 kDa.

In particular, the preparation of the invention comprises isoforms having pI values of 6.8, 7.1 and 7.6.

The invention also relates to a method of preparing "plantified" carbohydrate units of human and other vertebrate glycoproteins, wherein fucose units as well as GlcNAc- α 1,3-fucosyl transferase encoded by an above-described DNA molecule are admixed to a sample that comprises a carbohydrate unit or a glycoprotein, respectively, so that fucose in α 1,3-position will be bound by the GlcNAc- α 1,3-fucosyl transferase to the carbohydrate unit or to the glycoprotein, respectively. By the method according to the invention for cloning GlcNAc- α 1,3-fucosyl transferase it is possible to produce large amounts of purified enzyme. To obtain a fully active transferase, suitable reaction conditions are provided. It has been shown that the transferase has a particularly high activity at a pH of approximately 7, if 2-(N-morpholino)-ethane sulfonic acid-HCl is used as the buffer. In the presence of bivalent cations, in particular Mn^{2+} , the activity of the recombinant transferase is enhanced. The carbohydrate unit is admixed to the sample either in unbound form or bound to a protein. The recombinant transferase is active for both forms. The invention will be explained in more detail by way of the following examples and drawing figures to which, of course, it shall not be restricted.

In detail, in the drawings,
Figs. 1a and 1b show, as curves, the measured amounts of protein and the measured enzyme activity in the individual fractions of the eluate;
Fig. 2 shows an electrophoresis gel analysis of GlcNAc- α 1,3-fucosyl transferase;
Fig. 3 shows the result of the isoelectric focussing and the measured transferase activity of the individual isoforms;
Fig. 4 shows the N-terminal sequences of 4 tryptic peptides 1-4 as well as the DNA sequence of three primers, S1, A2 and A3;
Figs. 5a and 5b show the cDNA sequence of α 1,3-fucosyl transferase;
Figs. 6a and 6b show the amino acid sequence of α 1,3-fucosyl transferase derived therefrom;
Fig. 7 is a schematic representation of the α 1,3-fucosyl transfe-

rase as well as the hydrophobicity of the amino acid residues; Fig. 8 shows a comparison of the conserved motifs of various fucosyl transferases; Fig. 9 shows a comparison of the fucosyl transferase activity of insect cells transfected with the $\alpha 1,3$ -fucosyl transferase gene with that of a negative control; Figs. 10a and 10b show structures of different acceptors of the $\alpha 1,3$ -fucosyl transferase; Figs. 11 and 12 show mass spectra; and Fig. 13 shows the result of a HPLC.

Example 1:

Isolation of the core- $\alpha 1,3$ -fucosyl transferase

All the steps were carried out at 4°C. Mung bean seedlings were homogenized in a mixer, 0.75 volumes of extraction buffer being used per kg of beans. Subsequently, the homogenate was filtered through two layers of cotton fabric, and the filtrate was centrifuged for 40 min at 30000xg. The supernatant was discarded, and the pellet was extracted with solution buffer over night with continuous stirring. Subsequent centrifugation at 30000xg for 40 min yielded the triton extract.

The triton extract was purified as follows:

Step 1: The triton extract was applied to a microgranular diethyl amino ethyl cellulose anion exchanger DE52 cellulose column (5x28 cm) from Whatman, which previously had been calibrated with buffer A. The non-bound fraction was further treated in step 2.

Step 2: The sample was applied to an Affi-Gel Blue column (2,5x32) column calibrated with buffer A. After washing of the column with this buffer, adsorbed protein was eluted with buffer A comprising 0.5 M NaCl.

Step 3: After dialysis of the eluate from step 2 against buffer B, it was applied to an S-Sepharose column calibrated with the same buffer. Bound protein was eluted with a linear gradient of from 0 to 0.5 M NaCl in buffer B. Fractions with GlcNAc- $\alpha 1,3$ -fucosyl transferase were pooled and dialyzed against buffer C.

Step 4: The dialyzed sample was applied to a GnGn-Sepharose column calibrated with buffer C. The bound protein was eluted with buffer C comprising 1 M NaCl instead of $MnCl_2$.

Step 5: Subsequently, the enzyme was dialyzed against buffer

D and applied to a GDP-Hexanolamine-Sepharose column. After having washed the column with buffer D, the transferase was eluted by substituting $MgCl_2$ and NaCl with 0.5 mM GDP. Active fractions were pooled, dialyzed against 20 mM Tris-HCl buffer, pH 7.3, and lyophilized.

The enzymatic activity of the GlcNAc- α 1,3-fucosyl transferase was determined by using GnGn peptide and GDP-L-[U- ^{14}C]-fucose at substrate concentrations of 0.5 and 0.25 each, in the presence of 2-(N-morpholino)ethanesulfonic acid-HCl buffer, Triton X-100, $MnCl_2$, GlcNAc and AMP (according to Staudacher et al., 1998, Glycoconjugate J. 15, 355-360; Staudacher et al., 1991, Eur. J. Biochem. 199, 745-751).

Protein concentrations were determined by aid of the bicinchoninic acid method (Pierce) or, in the final steps of enzyme purification, by means of amino acid analysis (Altmann 1992, Anal. Biochem. 204, 215-219).

In Figs. 1a and 1b, the measured amounts of protein and the measured enzyme activity in the individual fractions of the eluate are illustrated as curves. Fig. 1a shows the above-described separation on the S-Sepharose column, Fig. 1b shows the separation on the GnGn-Sepharose column, the circle representing protein, the black, full circle representing GlcNAc- α 1,3-fucosyl transferase, and the square illustrating N-acetyl- β -glucosaminidase. One U is defined as that amount of enzyme which transfers 1 mmol of fucose onto an acceptor per minute.

Table 1 shows the individual steps of transferase purification.

Table 1

Purification step	Total protein	Total activity	Specific activity	Purification factor	Yield
	mg	mU	mU/mg	-fold	%
Triton X-100 extract	91500	4846	0.05	1	100
DE52	43700	4750	0.10	2	98.0
Affigel Blue	180.5	4134	23	460	85.3
S-Sepharose	8.4	3251	390	7800	67.1
GnGn-Sepharose	0.13 ¹	1044	8030	160000	21.5
GDP-Hexanolamine- Sepharose	0.02 ¹	867	43350	867000	17.9

¹determined by amino acid analysis

Extraction buffer:

0.5 mM Dithiothreitol
1 mM EDTA
0.5% Polyvinyl polypyrrolidone
0.25 M Sucrose
50 mM Tris-HCl buffer, pH 7.3

Solution buffer:

0.5 mM Dithiothreitol
1 mM EDTA
1.5% Triton X-100
50 mM Tris-HCl, pH 7.3

Buffer A:

25 mM Tris-HCl buffer, pH 7.3, comprising:
0.1% Triton X-100 and
0.02% NaN₃

Buffer B:

25 mM Na citrate buffer, pH 5.3, comprising:
0.1% Triton X-100 and
0.02% NaN₃

Buffer C:

25 mM Tris-HCl buffer, pH 7.3, comprising:
5 mM MnCl₂nd
0.02% NaN₃

Buffer D:

25 mM Tris-HCl, pH 7.3, comprising:
10 mM $MgCl_2$
0.1 M NaCl, and
0.02% NaN_3

Example 2:

SDS-PAGE and isoelectric focussing

An SDS-PAGE was carried out in a Biorad Mini-protean cell on gels with 12.5% acrylamide and 1% bisacrylamide. The gels were stained either with Coomassie Brilliant Blue R-250 or Silver. Isoelectric focussing of the fucosyl transferase was carried out on prefabricated gels having a pI range of between 6-9 (Servalyt precotes 6-9, Serva). The gels were stained with silver according to the producer's protocol. For the two-dimensional electrophoresis, lanes were cut out of the focussing gel, treated with S-alkylating reagents and SDS and subjected to an SDS-PAGE, as described above.

Fig. 2 shows the illustration of an electrophoresis gel of GlcNAc- α 1,3-fucosyl transferase, the two-dimensional electrophoresis being indicated on the left-hand side, and the one-dimensional SDS-PAGE being illustrated on the right-hand side. The lane denoted by A is a standard, the lane denoted by B is the GlcNAc- α 1,3-fucosyl transferase from the GnGn-Sepharose column, and the lane denoted by C is the "purified" GlcNAc- α 1,3-fucosyl transferase, i.e. the fraction of the GDP Hexanolamine Sepharose column. The two bands at 54 and 56 kDa represent isoforms of the transferase.

Fig. 3 shows the result of the isoelectric focussing. Lane A was stained with silver, on lane B, the activity of the transferase isoforms was tested. The activity is indicated as % fucose which had been transferred from GDP-fucose onto the substrate.

Example 3:

Peptide sequencing

For sequencing of the protein, bands were cut out of the Coomassie-stained SDS-Polyacrylamide gel, carboxyamido-methylated and cleaved with trypsin according to Görg et al. 1988, Electrophoresis, 9, 681-692. The tryptic peptides were separated with the reverse phase HPLC on a 1.0x250 mm Vydac C18 at 40°C at a flow rate of 0.05 ml/min, wherein a HP 1100 apparatus (Hewlett-

Packard) was used. The isolated peptides were separated with a Hewlett-Packard G1005 A Protein Sequencing System according to the producer's protocol. Furthermore, the peptide mixture was analyzed by Ingel digestion with MALDI-TOF MS (see below). Fig. 4 shows the N-terminal sequences of 4 tryptic peptides 1-4 (SEQ ID NO: 5-8). Departing from the first three peptides, primers S1, A2 and A3 were prepared (SEQ ID NO: 9-11).

Example 4:

RT-PCR and cDNA cloning

The entire RNA was isolated from a 3-day-old mung bean hypocotyl, wherein the SV Total RNA Isolating System of Promega was used. To prepare the first strand cDNA, the entire RNA was incubated for 1 h at 48°C with AMV reverse transcriptase and oligo(dT) primers, wherein the Reverse Transcription System of Promega was used. The first strand cDNA was subjected to a PCR, wherein a combination of sense and antisense primers was used: To 10 µl of the reverse transcription reaction mixture, the following was added:

50 µl with 0.1 mmol of each primer, 0.1 mM dNTPs, 2 mM MgCl₂, 10 mM Tris-HCl buffer, pH 9.0, 50 mM KCl and 0.1% Triton X-100. After a first denaturing step at 95°C for 2 min, 40 cycles of 1 min at 95°C, 1 min at 49°C and 2 min at 72°C were passed. The last extension step was carried out at 72°C for 8 min. PCR products were subcloned into the pCR2.1 vector, with the TA Cloning Kit of Invitrogen being used, and sequenced. The products of this PCR were two DNA fragments with lengths of 744 bp and 780 bp, both DNA fragments having the same 5'-end (cf. also Fig. 7). Starting from these two DNA fragments, the missing 5' and 3' regions of the cDNA were obtained by 5' and 3' rapid amplification of cDNA ends (RACE), wherein the RACE Kit of Gibco-BRL was used. As the antisense primer, the universal amplification primer of the kit, and as the sense primer, either 5'-CTGGAAGTGTCCCTGTGGTT-3' (SEQ ID NO: 12) or 5'-AGTGCACTAGAGGGCCAGAA-3' (SEQ ID NO: 13) were used. As the sense primer, also the shortened anchor primer of the kit, and as the antisense primer, 5'-TTTCGAGCACCA-CAATTGGAAAT-3' (SEQ ID NO: 14) or 5'-GAATGCAAAGACGGCAGATGAAT-3' (SEQ ID NO: 15) were used.

The PCR was carried out with an annealing temperature of 55°C and under the above-described conditions. The 5' and 3' RACE products were subcloned into the pCR2.1 vector and sequenced: The se-

quences of the subcloned fragments were sequenced by means of the dideoxynucleotide method (ABI PRISM Dye Terminator Cycle Sequencing Ready reaction Kit and ABI PRISM 310 Genetic analyser (Perkin Elmer)). T7 and M13 forward primers were used for the sequencing of the products cloned into vector pCR2.1. Both strands of the coding region were sequenced by the Vienna VBC Genomics-Sequencing Service, infrared-labelled primers (IRD700 and IRD800) and an LI-COR Long Read IR 4200 Sequencer (Lincoln, NE) being used.

Figs. 5a and 5b show the entire cDNA which has a size of 2198 bp and an open reading frame of 1530 bp (SEQ ID NO: 1). The open reading frame (start codon at base pairs 211-213, stop codon at base pairs 1740-1743) codes for a protein of 510 amino acids having a molecular weight of 56.8 kDa and a theoretical pI value of 7.51.

Figs. 6a and 6b show the cDNA-derived amino acid sequence of the GlcNAc- α 1,3-fucosyl transferase (SEQ ID NO: 2). Sites for the asparagine-bound glycosylation are at Asn346 and Asn429.

In Fig. 7, the schematic GlcNAc- α 1,3-fucosyl transferase-cDNA (top) and the derived hydrophobicity index of the encoded protein (bottom) are illustrated, a positive hydrophobicity index meaning an increased hydrophobicity. Therebetween, the sizes of the two above-indicated PCR products are shown in relationship to the complete cDNA. The coding region is illustrated by the beam, "C" coding for the postulated cytoplasmatic region, T for the postulated transmembrane region, and G for the postulated Golgi lumen catalytic region of transferase. The analysis of the DNA sequence by "TMpred" (from EMBnet, Switzerland) gave an assumed transmembrane region between Asn36 and Gly54. The C-terminal region of the enzyme probably comprises the catalytic region and consequently should point into the lumen of the Golgi apparatus. According to this, this transferase seems to be a type II transmembrane protein like all the hitherto analyzed glycosyl transferases which are involved in glycoprotein biosynthesis (Joziasse, 1992, Glycobiology 2, 271-277). The gray regions represent the four tryptic peptides, the hexagons represent the potential N-glycosylation sites. A BLASTP search in all data banks accessible via NCBI showed a similarity between the GlcNAc- α 1,3-fucosyl transferase and other α 1,3/4-fucosyl transferases, e.g. human fucosyl transferase VI. At 18-21% (examined by SIM-LALN-

VIEW, Expanse, Switzerland), the total similarity was beyond any significance. Nevertheless, a sequence range of 35 amino acids (SEQ ID NO: 4) shows a strikingly high homology to other α 1,3/4-fucosyl transferases (Fig. 8). This sequence region is located between Glu267 and Pro301 of SEQ ID NO: 2.

Example 5:

Expression of recombinant GlcNAc- α 1,3-fucosyl transferase in insect cells

The encoding region of the assumed GlcNAc- α 1,3-fucosyl transferase including cytoplasmatic and transmembrane region was amplified with the forward primer 5'-CGGCGGATCCGCAATTGAATGATG-3' (SEQ ID NO: 16) and reverse primer 5'-CCGGCTGCAGTACCATTAGCGCAT-3' (SEQ ID NO: 17) by means of the Expand High Fidelity PCR System of Boehringer Mannheim. The PCR product was double-digested with PstI and BamHI and subcloned in alkaline phosphatase-treated baculovirus transfer vector pVL1393 which previously had been digested with PstI and BamHI. To ensure a homologous recombination, the transfer vector was co-transfected with Baculo Gold viral DNA (PharMingen, Sand Diego, CA) in Sf9 insect cells in IPL-41 Medium with lipofectin. After an incubation of 5 days at 27°C, various volumes of the supernatant with the recombinant virus were used for infecting the Sf21 insect cells. After an incubation of 4 days at 27°C in IPL-41 Medium with 5% FCS, the Sf1 cells were harvested and washed 2x with phosphate-buffered saline solution. The cells were resuspended in 25 mM Tris HCl buffer, pH 7.4, with 2% Triton X-100 and broken up by sonication on ice.

Example 6:

Assay for GlcNAc- α 1,3-fucosyl transferase activity

The homogenate and the cell supernatant were assayed for GlcNAc- α 1,3-fucosyl transferase. Blind samples were carried out with recombinant baculovirus which codes for the tobacco-GlcNAc-transferase I (Strasser et al., 1999, Glycobiology, in the process of printing).

Fig. 9 shows the measured enzyme activity of the recombinant GlcNAc- α 1,3-fucosyl transferase as well as of the negative control. At best, the enzyme activity of the cotransfected cells and their supernatant was 30x higher than that of the negative controls. This endogenous activity which is measurable in the absence of the recombinant transferase, substantially comes from the insect- α 1,6-fucosyl transferase and only a low percentage

thereof comes from the GlcNAc- α 1,3-fucosyl transferase. Accordingly, the increase in the GlcNAc- α 1,3-fucosyl transferase coming from the recombinant baculoviruses is far more than the 100-fold. The enzyme exhibited a broad maximum activity around a pH of 7.0, if the activity was measured in 2-(N-morpholino)-ethanesulfonic acid-HCl buffer. As is apparent in Table 2, the addition of bivalent cations, in particular Mn^{2+} , enhances the activity of the recombinant transferase.

Table 2

Additive (conc. 10 mM)	Relative Activity (Acceptor: GnGn-peptide)

	%
none	21
EDTA	18
$MnCl_2$	100
$CaCl_2$	82
$MgCl_2$	52
$CdCl_2$	44
$CoCl_2$	35
$CuCl_2$	3
$NiCl_2$	24
$ZnCl_2$	0.6

Table 3 shows that among the acceptors used, the GnGn-peptide exhibits the highest incorporation rates under standard test conditions, followed closely by GnGnF⁶peptide and M5Gn-Asn. A transfer to the MM peptide could not be found, which MM peptide does not comprise the reducing GlcNAc-end at the 3-bound mannose. This structure seems to be necessary for the core fucosyl transferase. The recombinant transferase, moreover, was inactive relative to the acceptors commonly used, the α ,3/4-fucosyl transferases used for determining the blood groups, which transfer the fucose to GlcNAc at the non-reducing ends of oligosaccharides. The apparent K_m -values for the acceptor substrate GnGn peptide, GnGnF⁶peptide, M5Gn-Asn, and for the donor substrate GDP-fucose, were assessed to be 0.19, 0.13, 0.23 and 0.11, respectively. The structures of the molecules are illustrated in Figs. 10a and 10b.

Table 3

Acceptor Substrate	Rel. Activity	K _m -Value
	%	mM
GnGn-peptide	100	0.19
GnGnF ⁶ -peptide	87	0.13
M5Gn-Asn	71	0.23
MM-peptide	0	
Galβ-4GlcNAc	0	
Galβ1-3GlcNAc	0	
Galβ1-3GlcNAcβ1-3Galβ1-4Glc	0	

Example 7:

Mass spectrometry of the fucosyl transferase product

Dabsylated GnGn hexapeptide (2 nmol) was incubated with the insect cell homogenate comprising the recombinant GlcNAc- α ,3-fucosyl transferase (0.08 mU) in the presence of non-radioactive GDP-L-fucose (10 nmol), 2 (N-morpholino)-ethanesulfonic acid-HCl buffer, Triton X-100, MnCl₂, GlcNAc and AMP. A negative control was carried out with a homogenate of the infected insect cells for the blind samples. The samples were incubated for 16 h at 37°C and analyzed by means of MALDI TOF mass spectrometry. Mass spectrometry was performed on a DYNAMO (Thermo BioAnalysis, Santa Fe, NM), a MALDI-TOF MS which is capable of dynamic extraction (synonym for late extraction). Two types of sample matrix preparations were used: peptides and dabsylated glycopeptides were dissolved in 5% formic acid, and aliquots were applied to the target, air-dried, and covered with 1% α -cyano-4-hydroxy cinnamic acid. Pyridyl-aminated glycans, reduced oligosaccharides and non-derivatized glycopeptides were diluted with water, applied to the target and air-dried. After addition of 2% 2,5-dihydroxy benzoic acid, the samples were immediately dried by applying a vacuum.

Fig. 11 shows the mass spectrum of these samples, A being the negative control: The main peak (S) shows the Dabsyl-Val-Gly-Glu-(GlcNAc₄Man₃)Asn-Arg-Thr substrate, the calculated [M+H]⁺ value being 2262.3. This substrate also appears as sodium addi-

tion product and as smaller ion which has been formed by fragmentation of the Azo function of the Dabsyl group, at (S*). A small product amount (P, $[M+H]^+ = 2408.4$) is a consequence of the endogenous $\alpha 1,6$ -fucosyl transferase. The peak at $m/z = 2424.0$ shows the incomplete de-galactosylation of the substrate. The mass spectrum B shows the sample with recombinant $\alpha 1,3$ -fucosyl transferase. The main peak (P) represents the fucosylated product, (P*) its fragmented ion.

In addition, aliquots of both samples were mixed with each other so as to obtain similar concentrations of substrate and product (sample A). This mixture was diluted with 0.1 M ammonium acetate, pH 4.0, comprising 10 mU of N-glycosidase A (sample B), or with 50 mM Tris/HCl, pH 8.5, comprising 100 mU (1 U hydrolyses 1 mmol of substrate per min) of N-glycosidase F (sample C). After 2 and 20 h, small aliquots of these mixtures were taken and analyzed by means of MALDI-TOF MS.

In Fig. 12, the three mass spectra of samples A, B and C are illustrated. The undigested sample A shows two main peaks: the substrate at 2261.4 m/z , and the fucosylated product at 2407.7 m/z . The middle curve shows the mass spectrum of sample B, treated with N-glycosidase A, which hydrolyses both glycopeptides. The peak at 963.32 constitutes the deglycosylated product. The lower curve shows the mass spectrum of sample C. The N-glycosidase F is not able to hydrolyse $\alpha 1,3$ -fucosylated substrates, so that the spectrum has the peak at 2406.7 m/z of the fucosylated product, whereas the peak of the hydrolysed substrate appears at 963.08 m/z .

Example 8:

HPLC-analysis of the pyridyl-aminated fucosyl transferase product

The two above-described samples (fucosylated product and negative control) were digested with N-glycosidase A. The oligosaccharides obtained were pyridyl-aminated and analysed by means of reverse phase HPLC (Wilson et al., 1998, glycobiology 8, 651-661; Kubelka et al., 1994, Arch. Biochem. Biophys. 308, 148-157; Hase et al., 1984, J. Biochem. 95, 197-203).

In Fig. 13, the top diagram B represents the negative control, wherein in addition to the residual substrate (GnGn-peptide) $\alpha 1,6$ -fucosylated product is visible. A has a peak at a substan-

tially shorter retention time, which is specific of reducing fucose bound to GlcNAc- α 1,3.

In the bottom diagram, the isolated transferase product prior to (curve A) and following (curve B) digestion by N-acetyl- β glucosaminidase was compared with MMF³ honeybee phospholipase A₂ (curve C).

1000
900
800
700
600
500
400
300
200
100
0
1000
900
800
700
600
500
400
300
200
100
0
1000
900
800
700
600
500
400
300
200
100
0
1000
900
800
700
600
500
400
300
200
100
0

SEQUENCE LISTING

<110> Altmann Dr., Friedrich

<120> alpha 1,3-fucosyltransferase R35063

<130> fucosyltransferase gene

<140>

<141>

<160> 17

<170> PatentIn Ver. 2.1

<210> 1

<211> 2198

<212> DNA

<213> plant

<400> 1

```

actaaactcaa acgctgcatt ttcttttttc tttcagggaa ccatccaccc ataacaacaa 60
aaaaaacacac agcaagctgt gtttttttta tcgttctttt tctttaaaca agcaccacca 120
tcatggaatc gtgctcataa cgccaaaatt ttccatttcc ctttgatttt tagttttatt 180
tgcggaattg gcagttgggg gcgcaattga atgatgggtc tgttgacgaa tcttcgaggc 240
tcgagaacag atggtgccca acaagacagc ttaccctgtt tggctccggg aggcaacca 300
aagaggaaat ggagcaatct aatgcctctt gttgtgtccc ttgtggtcat cgcggagatc 360
gcgtttctgg gtaggttgga tatggccaaa aacgccacca tggttgactc cctcgtgtac 420
ttcttctacc gctctcgagc ggtcgttgaa ggtgacgatt tggggttggg tttggtggct 480
tctgacgcga attctgaatc gtatagttgt gaggaatggt tggagaggga ggtagctgtc 540
acgtatttca ggggcttttc caaagagcct atttttgttt ctggagctga tcaggagtgg 600
aagtcgtggt cggttgggat taaatttggg tttagtgggg atagaagcc agatgccga 660
tttgggttac ctcaaccaag tggaaacgct agcattctgc gatcaatgga atcagcagaa 720
tactatgctg agaacaatat tgccatggca agacggaggg gatataacat cgtaatgaca 780
accagtctat ctccgtagtg tctgttgga tatttttcat ggcctgagta tgatatgatg 840
gcaccagtgc agccgaaaaa tgaagctgct cttgcagctg ctttcatctc caattgtggt 900
gtcgaaaatt tccggttgca agctcttgag gcccttgaaa aatcaaacat caaaattgat 960
tcttatgggt gttgtcacag gaaccgtgat ggaagagtga acaaagtgga agccctgaag 1020
cactacaaat ttagtcttag gtttgaaaat tcgaatgagg aagattatgt aactgaaaaa 1080
ttcttccaat cccttgttgc tggaaactgtc cctgtggttg ttggtgtctc aaatattcag 1140
gactttgtct cttctcctgg ttcaatttta catattaaag agatagagga tgttgagtct 1200
gttgcaaaaga ccatgagata tctagcagaa aatcccgaag catataatca atcattgagg 1260
tggaaagtat aggggtccat tgactccttc aaggcccttg tggatatggc agctgtgcat 1320
tcactgtgcc gtctttgcat tcaattggcc acagtgaata gagagaagga agaaaataat 1380
ccaagcctta agagacgtcc ttgcaagtgc actagagggc cagaaacccg atatacatc 1440
tatgtcagag aaaggggaag gtttgagatg gactccattt acctgaggtc tagcaattta 1500
actctgaatg ctgtgaaggc tgctgttggt ttgaagtcca catccctgaa tcttgtgcct 1560
gtatggaaga ctgaaaggcc tgaagttata agagggggga gtgctttaa atctctacaa 1620

```

atatacccaa ttggcttgac acagagacaa gctctttata ccttcagctt caaaggtgat 1680
gctgatttca ggagtcactt ggagaacaat ccttggtcca agtttgaagt ctttttgtg 1740
tagcatgcgc taaatggtac ctctgctcta cctgaattag cttcacttag ctgagcacta 1800
gctagagttt taggaatgag tatggcagtg aatatggcat ggctttatct atgcctagtt 1860
tcttggccaa ctcatgtatg ttttgtataa gacatcacac ttaattttaa aacttggttc 1920
tgtagaagtg caaatccata tttaatgctt agttttatgt ctcttatctg atcatctaga 1980
agtcacagtt cttgtatatt gtgagtga aaactgaaatct aatagaagga tcagatgttt 2040
cactcaagac acattattac ttcatgttgt tttgatgac tcgagctttt ttagtgtctg 2100
gaactgtccc tgggttttga gcacctgtta ttgcttcagt gttactgtcc agtgggtatc 2160
gtttttgacc tctaaaaaaa aaaaaaaaaa aaaaaaaaaa 2198

<210> 2

<211> 510

<212> PRT

<213> plant

<400> 2

Met Met Gly Leu Leu Thr Asn Leu Arg Gly Ser Arg Thr Asp Gly Ala
1 5 10 15

Gln Gln Asp Ser Leu Pro Val Leu Ala Pro Gly Gly Asn Pro Lys Arg
20 25 30

Lys Trp Ser Asn Leu Met Pro Leu Val Val Ala Leu Val Val Ile Ala
35 40 45

Glu Ile Ala Phe Leu Gly Arg Leu Asp Met Ala Lys Asn Ala Ala Met
50 55 60

Val Asp Ser Leu Ala Asp Phe Phe Tyr Arg Ser Arg Ala Val Val Glu
65 70 75 80

Gly Asp Asp Leu Gly Leu Gly Leu Val Ala Ser Asp Arg Asn Ser Glu
85 90 95

Ser Tyr Ser Cys Glu Glu Trp Leu Glu Arg Glu Asp Ala Val Thr Tyr
100 105 110

Ser Arg Gly Phe Ser Lys Glu Pro Ile Phe Val Ser Gly Ala Asp Gln
115 120 125

Glu Trp Lys Ser Cys Ser Val Gly Cys Lys Phe Gly Phe Ser Gly Asp
130 135 140

Arg Lys Pro Asp Ala Ala Phe Gly Leu Pro Gln Pro Ser Gly Thr Ala
145 150 155 160

Ser Ile Leu Arg Ser Met Glu Ser Ala Glu Tyr Tyr Ala Glu Asn Asn

	165		170		175
Ile Ala Met Ala Arg Arg Arg Gly Tyr Asn Ile Val Met Thr Thr Ser					
	180		185		190
Leu Ser Ser Asp Val Pro Val Gly Tyr Phe Ser Trp Ala Glu Tyr Asp					
	195		200		205
Met Met Ala Pro Val Gln Pro Lys Thr Glu Ala Ala Leu Ala Ala Ala					
	210		215		220
Phe Ile Ser Asn Cys Gly Ala Arg Asn Phe Arg Leu Gln Ala Leu Glu					
225		230		235	240
Ala Leu Glu Lys Ser Asn Ile Lys Ile Asp Ser Tyr Gly Gly Cys His					
	245		250		255
Arg Asn Arg Asp Gly Arg Val Asn Lys Val Glu Ala Leu Lys His Tyr					
	260		265		270
Lys Phe Ser Leu Ala Phe Glu Asn Ser Asn Glu Glu Asp Tyr Val Thr					
	275		280		285
Glu Lys Phe Phe Gln Ser Leu Val Ala Gly Thr Val Pro Val Val Val					
	290		295		300
Gly Ala Pro Asn Ile Gln Asp Phe Ala Pro Ser Pro Gly Ser Ile Leu					
305		310		315	320
His Ile Lys Glu Ile Glu Asp Val Glu Ser Val Ala Lys Thr Met Arg					
	325		330		335
Tyr Leu Ala Glu Asn Pro Glu Ala Tyr Asn Gln Ser Leu Arg Trp Lys					
	340		345		350
Tyr Glu Gly Pro Ser Asp Ser Phe Lys Ala Leu Val Asp Met Ala Ala					
	355		360		365
Val His Ser Ser Cys Arg Leu Cys Ile His Leu Ala Thr Val Ser Arg					
	370		375		380
Glu Lys Glu Glu Asn Asn Pro Ser Leu Lys Arg Arg Pro Cys Lys Cys					
385		390		395	400
Thr Arg Gly Pro Glu Thr Val Tyr His Ile Tyr Val Arg Glu Arg Gly					
	405		410		415
Arg Phe Glu Met Glu Ser Ile Tyr Leu Arg Ser Ser Asn Leu Thr Leu					

420

425

430

Asn Ala Val Lys Ala Ala Val Val Leu Lys Phe Thr Ser Leu Asn Leu
435 440 445

Val Pro Val Trp Lys Thr Glu Arg Pro Glu Val Ile Arg Gly Gly Ser
450 455 460

Ala Leu Lys Leu Tyr Lys Ile Tyr Pro Ile Gly Leu Thr Gln Arg Gln
465 470 475 480

Ala Leu Tyr Thr Phe Ser Phe Lys Gly Asp Ala Asp Phe Arg Ser His
485 490 495

Leu Glu Asn Asn Pro Cys Ala Lys Phe Glu Val Ile Phe Val
500 505 510

<210> 3

<211> 105

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:cDNA

<400> 3

gaagccctga agcactacaa atttagctta gcgtttgaaa attcgaatga ggaagattat 60
gtaactgaaa aattcttcca atcccttggt gctggaactg tccct 105

<210> 4

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:peptide

<400> 4

Glu Ala Leu Lys His Tyr Lys Phe Ser Leu Ala Phe Glu Asn Ser Asn
1 5 10 15

Glu Glu Asp Tyr Val Thr Glu Lys Phe Phe Gln Ser Leu Val Ala Gly
20 25 30

Thr Val Pro

<210> 5
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide

<400> 5
Lys Pro Asp Ala Xaa Phe Gly Leu Pro Gln Pro Ser Thr Ala Ser
1 5 10 15

<210> 6
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide

<400> 6
Pro Glu Thr Val Tyr His Ile Tyr Val Arg
1 5 10

<210> 7
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide

<400> 7
Met Glu Ser Ala Glu Tyr Tyr Ala Glu Asn Asn Ile Ala
1 5 10

<210> 8
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide

<400> 8

Gly Arg Phe Glu Met Glu Ser Ile Tyr Leu

1

5

10

<210> 9

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DNA

<400> 9

gcngartayt aygcngaraa yaayathgc

29

<210> 10

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DNA

<400> 10

crtadatrtg rtanacngty tc

22

<210> 11

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DNA

<400> 11

tadatnswyt ccatytcraa

20

<210> 12

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DNA

<400> 12

ctggaactgt cctctgtggtt

20

<210> 13
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:DNA

<400> 13
agtgcactag agggccagaa 20

<210> 14
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:DNA

<400> 14
ttcgagcacc acaattggaa at 22

<210> 15
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:DNA

<400> 15
gaatgcaaag acggcagat gaat 24

<210> 16
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:DNA

<400> 16
cggcggatcc gcaattgaat gatg 24

<210> 17
<211> 25
<212> DNA

WHAT WE CLAIM IS:

1. A DNA molecule, characterized in that it comprises a sequence according to SEQ ID NO 1 with an open reading frame from base pair 211 to base pair 1740, or is at least 50% homologous with the above sequence, or hybridizes with the above sequence under stringent conditions, or comprises a sequence which has degenerated to the above DNA sequence due to the genetic code, with the sequence coding for a plant protein having fucosyl transferase activity or being complementary thereto.

2. A DNA molecule according to claim 1, characterized in that it codes for a protein having GlcNAc- α 1,3-fucosyl transferase activity, particularly core- α 1,3-fucosyl transferase activity.

3. A DNA molecule according to claims 1 or 2, characterized in that it is at least 70-80%, particularly preferably at least 95% homologous with the sequence according to SEQ ID NO 1.

4. A DNA molecule according to any one of claims 1 to 3, characterized in that it comprises 2150 to 2250, particularly 2198 base pairs.

5. A DNA molecule, characterized in that it comprises a sequence according to SEQ ID NO 3, or comprises a sequence which is at least 85%, particularly at least 95% homologous with the above sequence or hybridizes with the above sequence under stringent conditions or has degenerated to the above DNA sequence due to the genetic code.

6. A DNA molecule, characterized in that it comprises a partial sequence of a DNA molecule according to any one of claims 1 to 5 and has a size of 20 to 200, preferably 30 to 50 base pairs.

7. A DNA molecule according to any one of claims 1 to 6, characterized in that it is covalently associated with a detectable marker substance.

8. A biologically functional vector, characterized in that it comprises a DNA molecule according to any one of claims 1 to 7 or parts thereof of different length having at least 20 base pairs.

9. A biologically functional vector, characterized in that it comprises a DNA molecule according to any one of claims 1 to

7 or parts thereof of different length being inversely orientated with respect to the promotor.

10. A DNA molecule according to any one of claims 1 to 7, characterized in that said DNA sequence comprises a deletion, insertion and/or substitution mutation.

11. A DNA molecule coding for a ribozyme, characterized in that it has two sequence sections, each of which has a length of at least 10 to 15 base pairs and which are complementary to the sequence sections of a DNA molecule according to any one of claims 1 to 7 so that said ribozyme complexes and cuts the mRNA transcribed by a natural GlcNAc- α 1,3-fucosyl transferase DNA molecule.

12. A biologically functional vector, characterized in that it comprises a DNA molecule according to claims 10 or 11.

13. A method of preparing a cDNA comprising a DNA molecule according to any one of claims 1 to 5, characterized in that RNA is isolated from insect or plant cells, particularly from hypocotylous cells, and with said RNA a reverse transcription is effected after the addition of a reverse transcriptase and primers.

14. A method of cloning a GlcNAc- α 1,3-fucosyl transferase, characterized in that a DNA molecule according to any one of claims 1 to 5 is cloned into a vector subsequently transfected into a host cell or a host, with cell lines being obtained by means of selection and amplification of transfected host cells, which cell lines express the active GlcNAc- α 1,3-fucosyl transferase.

15. A method of preparing recombinant host cells, particularly plant or insect cells, or plants or insects, respectively, wherein the production of GlcNAc- α -1,3-fucosyl transferase is suppressed or completely stopped, characterized in that at least one of the vectors according to claims 8, 9 or 12 is inserted into said host cell, or plant or insect, respectively.

16. A method of preparing recombinant host cells, particularly plant or insect cells, or plants or insects, respectively, characterized in that the DNA molecule according to claim 10 is inserted into the genome of said host cell, or plant or insect, respectively, at the position of the non-

mutated, homologous sequence.

17. Recombinant plants or plant cells, characterized in that they are prepared according to a method according to claims 15 or 16 and that their GlcNAc- α 1,3-fucosyl transferase production is suppressed or completely stopped.

18. Recombinant insects or insect cells, characterized in that they are prepared according to a method according to claims 15 or 16 and that their GlcNAc- α 1,3-fucosyl transferase production is suppressed or completely stopped.

19. A PNA molecule, characterized in that it comprises a base sequence complementary to the sequence of a DNA molecule according to any one of claims 1 to 6 and partial sequences thereof.

20. A PNA molecule, characterized in that it comprises a base sequence corresponding to the sequence of a DNA molecule according to any one of claims 1 to 6 and partial sequences thereof.

21. A method of producing plants or insects, or cells, respectively, particularly plant or insect cells having blocked expression of GlcNAc- α 1,3-fucosyl transferase at the transcription or translation level, characterized in that PNA molecules according to claims 19 or 20 are inserted into the cells.

22. A method of producing recombinant glycoproteins, characterized in that the system according to claims 17 or 18 or plants or insects, or cells, respectively, which are prepared according to a method according to claim 21, is (are) transfected with the gene that expresses the glycoprotein so that the recombinant glycoproteins are expressed.

23. A method of producing recombinant human glycoproteins, characterized in that the system according to claims 17 or 18 or plants or insects, or cells, respectively, which are prepared according to a method according to claim 21, is (are) transfected with the gene that expresses the glycoprotein so that the recombinant glycoproteins are expressed.

24. A method of producing recombinant human glycoproteins for medical use, characterized in that the system according to claims 17 or 18 or plants or insects, or cells, respectively, which are prepared according to a method according to claim 21,

is (are) transfected with the gene that expresses the glycoprotein so that the recombinant glycoproteins are expressed.

25. Recombinant glycoproteins, characterized in that they are prepared according to the method according to claim 22 in plant or insect systems and that their peptide sequence has less than 50%, particularly less than 20%, particularly preferably 0% of α 1,3-bound fucose residues present in proteins expressed in non-fucosyl transferase reduced plant or insect systems.

26. Recombinant human glycoproteins, characterized in that they are prepared according to the method according to claim 23 in plant or insect systems and that their peptide sequence has less than 50%, particularly less than 20%, particularly preferably 0% of α 1,3-bound fucose residues present in proteins expressed in non-fucosyl transferase reduced plant or insect systems.

27. Recombinant human glycoproteins for medical use, characterized in that they are prepared according to the method according to claim 24 in plant or insect systems and that their peptide sequence has less than 50%, particularly less than 20%, particularly preferably 0% of α 1,3-bound fucose residues present in proteins expressed in non-fucosyl transferase reduced plant or insect systems.

28. A pharmaceutical composition, characterized in that it comprises recombinant glycoproteins according to any one of claims 25 to 27.

29. A method of selecting DNA molecules coding for a GlcNAc- α 1,3-fucosyl transferase, in a sample, characterized in that DNA molecules according to claim 7 are added to said sample, which molecules bind to the DNA molecules coding for a GlcNAc- α 1,3-fucosyl transferase.

30. A method according to claim 29, characterized in that said sample comprises genomic DNA of a plant or insect organism.

31. DNA molecules coding for a GlcNAc- α 1,3-fucosyl transferase, characterized in that they are selected according to the method according to claims 29 or 30 and are subsequently isolated from the sample.

32. A preparation of GlcNAc- α 1,3-fucosyl transferase cloned according to a method according to claim 14, characterized in

that it has isoforms having pI values of between 6.0 and 9.0, particularly between 6.8 and 8.2.

33. A preparation according to claim 32, characterized in that it has isoforms having pI values of 6.8, 7.1 and 7.6.

34. A method of preparing plantified carbohydrate units of human and other vertebrate glycoproteins, characterized in that to a sample comprising a carbohydrate unit or a glycoprotein, respectively, are added fucose units and GlcNAc- α 1,3-fucosyl transferase coded by a DNA molecule according to any one of claims 1 to 7 so that fucose is bound to said carbohydrate unit or said glycoprotein, respectively, at the α 1,3-position by said GlcNAc- α 1,3-fucosyl transferase.

200 1
200 2
200 3
200 4
200 5
200 6
200 7
200 8
200 9
200 10
200 11
200 12
200 13
200 14
200 15
200 16
200 17
200 18
200 19
200 20
200 21
200 22
200 23
200 24
200 25
200 26
200 27
200 28
200 29
200 30
200 31
200 32
200 33
200 34
200 35
200 36
200 37
200 38
200 39
200 40
200 41
200 42
200 43
200 44
200 45
200 46
200 47
200 48
200 49
200 50
200 51
200 52
200 53
200 54
200 55
200 56
200 57
200 58
200 59
200 60
200 61
200 62
200 63
200 64
200 65
200 66
200 67
200 68
200 69
200 70
200 71
200 72
200 73
200 74
200 75
200 76
200 77
200 78
200 79
200 80
200 81
200 82
200 83
200 84
200 85
200 86
200 87
200 88
200 89
200 90
200 91
200 92
200 93
200 94
200 95
200 96
200 97
200 98
200 99
200 100

Abstract:

A DNA molecule is provided which comprises a sequence according to SEQ ID NO: 1 having an open reading frame from base pair 211 to base pair 1740 or having at least 50% homology to the above-indicated sequence, or hybridizing with the above-indicated sequence under stringent conditions, or comprising a sequence which has degenerated to the above-indicated DNA sequence because of the genetic code, the sequence coding for a plant protein having fucosyltransferase activity or being complementary thereto.

1000
999
998
997
996
995
994
993
992
991
990
989
988
987
986
985
984
983
982
981
980
979
978
977
976
975
974
973
972
971
970
969
968
967
966
965
964
963
962
961
960
959
958
957
956
955
954
953
952
951
950
949
948
947
946
945
944
943
942
941
940
939
938
937
936
935
934
933
932
931
930
929
928
927
926
925
924
923
922
921
920
919
918
917
916
915
914
913
912
911
910
909
908
907
906
905
904
903
902
901
900
899
898
897
896
895
894
893
892
891
890
889
888
887
886
885
884
883
882
881
880
879
878
877
876
875
874
873
872
871
870
869
868
867
866
865
864
863
862
861
860
859
858
857
856
855
854
853
852
851
850
849
848
847
846
845
844
843
842
841
840
839
838
837
836
835
834
833
832
831
830
829
828
827
826
825
824
823
822
821
820
819
818
817
816
815
814
813
812
811
810
809
808
807
806
805
804
803
802
801
800
799
798
797
796
795
794
793
792
791
790
789
788
787
786
785
784
783
782
781
780
779
778
777
776
775
774
773
772
771
770
769
768
767
766
765
764
763
762
761
760
759
758
757
756
755
754
753
752
751
750
749
748
747
746
745
744
743
742
741
740
739
738
737
736
735
734
733
732
731
730
729
728
727
726
725
724
723
722
721
720
719
718
717
716
715
714
713
712
711
710
709
708
707
706
705
704
703
702
701
700
699
698
697
696
695
694
693
692
691
690
689
688
687
686
685
684
683
682
681
680
679
678
677
676
675
674
673
672
671
670
669
668
667
666
665
664
663
662
661
660
659
658
657
656
655
654
653
652
651
650
649
648
647
646
645
644
643
642
641
640
639
638
637
636
635
634
633
632
631
630
629
628
627
626
625
624
623
622
621
620
619
618
617
616
615
614
613
612
611
610
609
608
607
606
605
604
603
602
601
600
599
598
597
596
595
594
593
592
591
590
589
588
587
586
585
584
583
582
581
580
579
578
577
576
575
574
573
572
571
570
569
568
567
566
565
564
563
562
561
560
559
558
557
556
555
554
553
552
551
550
549
548
547
546
545
544
543
542
541
540
539
538
537
536
535
534
533
532
531
530
529
528
527
526
525
524
523
522
521
520
519
518
517
516
515
514
513
512
511
510
509
508
507
506
505
504
503
502
501
500
499
498
497
496
495
494
493
492
491
490
489
488
487
486
485
484
483
482
481
480
479
478
477
476
475
474
473
472
471
470
469
468
467
466
465
464
463
462
461
460
459
458
457
456
455
454
453
452
451
450
449
448
447
446
445
444
443
442
441
440
439
438
437
436
435
434
433
432
431
430
429
428
427
426
425
424
423
422
421
420
419
418
417
416
415
414
413
412
411
410
409
408
407
406
405
404
403
402
401
400
399
398
397
396
395
394
393
392
391
390
389
388
387
386
385
384
383
382
381
380
379
378
377
376
375
374
373
372
371
370
369
368
367
366
365
364
363
362
361
360
359
358
357
356
355
354
353
352
351
350
349
348
347
346
345
344
343
342
341
340
339
338
337
336
335
334
333
332
331
330
329
328
327
326
325
324
323
322
321
320
319
318
317
316
315
314
313
312
311
310
309
308
307
306
305
304
303
302
301
300
299
298
297
296
295
294
293
292
291
290
289
288
287
286
285
284
283
282
281
280
279
278
277
276
275
274
273
272
271
270
269
268
267
266
265
264
263
262
261
260
259
258
257
256
255
254
253
252
251
250
249
248
247
246
245
244
243
242
241
240
239
238
237
236
235
234
233
232
231
230
229
228
227
226
225
224
223
222
221
220
219
218
217
216
215
214
213
212
211
210
209
208
207
206
205
204
203
202
201
200
199
198
197
196
195
194
193
192
191
190
189
188
187
186
185
184
183
182
181
180
179
178
177
176
175
174
173
172
171
170
169
168
167
166
165
164
163
162
161
160
159
158
157
156
155
154
153
152
151
150
149
148
147
146
145
144
143
142
141
140
139
138
137
136
135
134
133
132
131
130
129
128
127
126
125
124
123
122
121
120
119
118
117
116
115
114
113
112
111
110
109
108
107
106
105
104
103
102
101
100
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

1/16

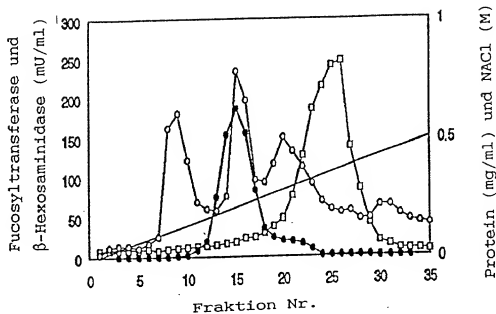


FIG.1 a

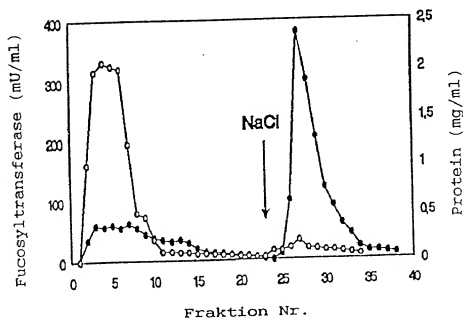


FIG.1 b

2/16

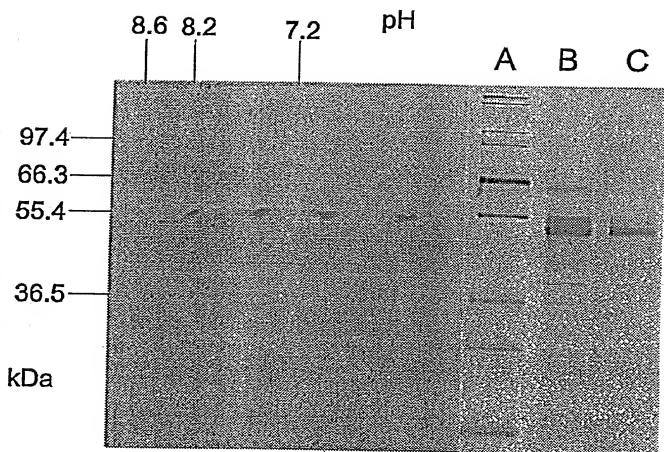
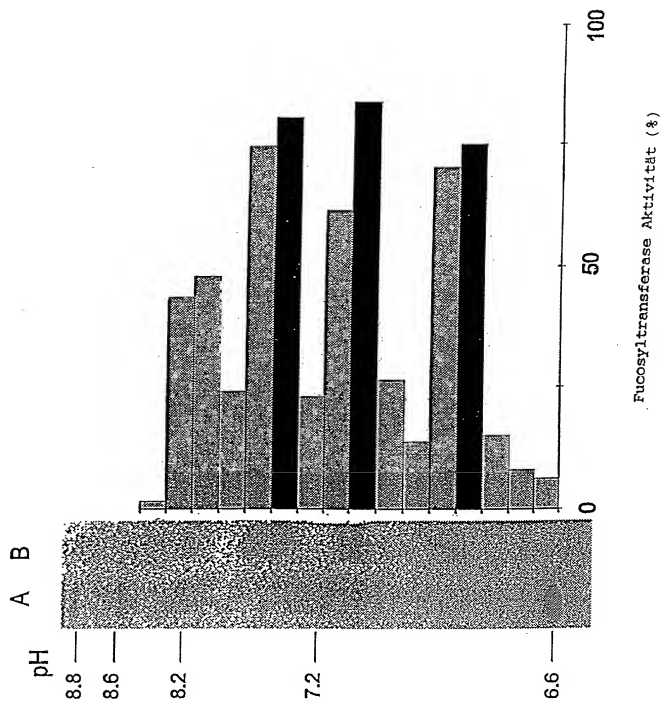


FIG.2

3/16

FIG.3



- 1 KPDA_xFGLPQPSTAS
- 2 PETVYHIYVR
- 3 MESAEYYAENNIA
- 4 GRFEMESIYL

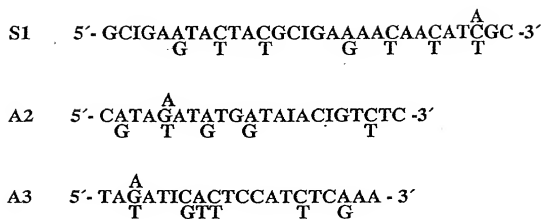


FIG.4

ACTAACTCAA ACGCTGCATT TTCTTTTTTC TTTCAGGGAA CCATCCACCC ATAACAACAA 60
 AAAAAACAAC AGCAAGCTGT GTTTTTTTTA TCGTTCCTTT TCTTTAAACA AGCACCCCCA 120
 TCATGGAATC GTGCTCATAA CGCCAAAATT TTCCATTTC CTTTGATTTT TAGTTIATTT 180
 TCGGGAATTG GCAGTTGGGG GCGCAATTGA ATGATGGGTC TGTTCACGAA TCTTCGAGGC 240
 TCGAGAACAG ATGGTGCCCA ACAAGACAGC TTACCCGTTT TGGCTCCGGG AGGCAACCCA 300
 AAGAGGAAAT GGAGCAATCT AATGCCTCTT GTTGTGCCCC TTGTGGTCAT CGCGGAGATC 360
 GCGTTTCTGG GTAGGTTGGA TATGGCCAAA AACGCCGCCA TG GTTGACTC CCTCGCTGAC 420
 TTCTTCTACC GCTCTCGAGC GGTGCTTGAA GGTGACGATT TGGGGTTGGG TTTGGTGGCT 480
 TCTGATCGGA ATTCTGAATC GTATAGTTGT GAGGAATGGT TGGAGAGGGA GGATGCTGTC 540
 ACGTATTCGA GGGGCTTTTC CAAAGAGCCT ATTTTGTITT CTGGAGCTGA TCAGGAGTGG 600
 AAGTCGTGTT CGGTTGGATG TAAATTTGGG TTTAGTGGGG ATAGAAAGCC AGATGCCGCA 660
 TTTGGGTTAC CTCAACCAAG TGGAACAGCT AGCATTCTGC GATCAATGGA ATCAGCAGAA 720
 TACTATGCTG AGAACAAATAT TGCCATGGCA AGACGGAGGG GATATAACAT CGTAATGACA 780
 ACCAGTCTAT CTTGGGATGT TCCTGTTGGA TATTTTTCAT GGGCTGAGTA TGATATGATG 840
 GCACCACTGC AGCCGAAAAC TGAAGCTGCT CTGCGAGCTG CTTTCATTTC CAATTGTGGT 900
 GCTCGAAATT TCCGGTIGCA AGCTCTTGAG GCCCTGAAA AATCAAACAT CAAAATTGAT 960
 TCTTATGGTG GTTGTACAGC GAACCGTAT GGAAGAGTGA ACAAAGTGA AGCCCTGAAG 1020
 CACTACAAAT TTAGCTTAGC GTTTGAAAAT TCGAATGAGG AAGATTATGT AACTGAAAAA 1080
 TTCTTCCAAT CCCTTGTTCG TGGAAGTGC CCTGTGGTTG TTGGTGTCC AAATATTGAG 1140

FIG.5 a

GACTTTGCTC	CTTCTCCTGG	TTCAATTTTA	CATATTAAAG	AGATAGAGGA	TGTTGAGTCT	1200
GTTCGAAAGA	CCATGAGATA	TCTAGCAGAA	AATCCCGAAG	CATATAATCA	ATCATTGAGG	1260
TGGAAGTATG	AGGGTCCATC	TGACTCCTTC	AAGGCCCTTG	TGGATATGGC	AGCTGTGCAT	1320
TCATCGTGGC	GTCTTTGCAT	TCACTTGGCC	ACAGTGAGTA	GAGAGAAGGA	AGAAAATAAT	1380
CCAAGCCTTA	AGAGACGTCC	TTGCAAGTGC	ACTAGAGGGC	CAGAAACCGT	ATATCATATC	1440
TATGTCAGAG	AAAGGGGAAG	GTTTGAGATG	GAGTCCATTT	ACCTGAGGTC	TAGCAATTTA	1500
ACTCTGAATG	CTGTGAAGGC	TGCTGTTGTT	TTGAAGTTCA	CATCCCTGAA	TCTTGTGCCT	1560
GTATGGAAGA	CTGAAAGGCC	TGAAGTTATA	AGAGGGGGGA	GTGCTTTAAA	ACTCTACAAA	1620
ATATACCCAA	TTGGCTTGAC	ACAGAGACAA	GCTCTTTATA	CCTTCAGCTT	CAGAGGTGAT	1680
GCTGATTTCA	GGAGTCACCT	GGAGAACAAT	CCTTGTGCCA	AGTTTGAAGT	CATTTTGTGT	1740
TAGCATGCGC	TAAATGGTAC	CTCTGCTCTA	CCTGAATTAG	CTTCACCTTAG	CTGAGCACTA	1800
GCTAGAGTTT	TAGGAATGAG	TATGGCAGTG	AATATGGCAT	GGCTTTATTT	ATGCCTAGTT	1860
TCTTGGCCAA	CTCATTGATG	TTTTGTATAA	GACATCACAC	TTTAATTTTA	AACTTGT TTC	1920
TGTAGAAGTG	CAAAICCATTA	TTAATGCTT	AGTTTTAGTG	CTCTTATCTG	ATCATCTAGA	1980
AGTCACAGTT	CTTGTATATT	GTGAGTGAAA	ACTGAAATCT	AATAGAAGGA	TCAGATGTTT	2040
CACTCAAGAC	ACATTATTAC	TTCATGTTGT	TTTGATGATC	TCGAGCTTTT	TTAGTGTCTG	2100
GAACTGTCCC	TGTGGTTTGA	GCACCTGTTA	TTGCTTCAGT	GTTACTGTCC	AGTGGTTATC	2160
GTTTTTGACC	TCTAAAAAAA	AAAAAAAAAA	AAAAAAAAAA			2198

FIG.5 b

7/16

Met Met Gly Leu Leu Thr Asn Leu Arg Gly Ser Arg Thr Asp Gly Ala
 1 5 10 15
 Gln Gln Asp Ser Leu Pro Val Leu Ala Pro Gly Gly Asn Pro Lys Arg
 20 25 30
 Lys Trp Ser Asn Leu Met Pro Leu Val Val Ala Leu Val Val Ile Ala
 35 40 45
 Glu Ile Ala Phe Leu Gly Arg Leu Asp Met Ala Lys Asn Ala Ala Met
 50 55 60
 Val Asp Ser Leu Ala Asp Phe Phe Tyr Arg Ser Arg Ala Val Val Glu
 65 70 75 80
 Gly Asp Asp Leu Gly Leu Gly Leu Val Ala Ser Asp Arg Asn Ser Glu
 85 90 95
 Ser Tyr Ser Cys Glu Glu Trp Leu Glu Arg Glu Asp Ala Val Thr Tyr
 100 105 110
 Ser Arg Gly Phe Ser Lys Glu Pro Ile Phe Val Ser Gly Ala Asp Gln
 115 120 125
 Glu Trp Lys Ser Cys Ser Val Gly Cys Lys Phe Gly Phe Ser Gly Asp
 130 135 140
 Arg Lys Pro Asp Ala Ala Phe Gly Leu Pro Gln Pro Ser Gly Thr Ala
 145 150 155 160
 Ser Ile Leu Arg Ser Met Glu Ser Ala Glu Tyr Tyr Ala Glu Asn Asn
 165 170 175
 Ile Ala Met Ala Arg Arg Arg Gly Tyr Asn Ile Val Met Thr Thr Ser
 180 185 190
 Leu Ser Ser Asp Val Pro Val Gly Tyr Phe Ser Trp Ala Glu Tyr Asp
 195 200 205
 Met Met Ala Pro Val Gln Pro Lys Thr Glu Ala Ala Leu Ala Ala Ala
 210 215 220
 Phe Ile Ser Asn Cys Gly Ala Arg Asn Phe Arg Leu Gln Ala Leu Glu
 225 230 235 240
 Ala Leu Glu Lys Ser Asn Ile Lys Ile Asp Ser Tyr Gly Gly Cys His

FIG.6 a

Arg Asn Arg Asp Gly Arg Val Asn Lys Val Glu Ala Leu Lys His Tyr
 260 265 270
 Lys Phe Ser Leu Ala Phe Glu Asn Ser Asn Glu Glu Asp Tyr Val Thr
 275 280 285
 Glu Lys Phe Phe Gln Ser Leu Val Ala Gly Thr Val Pro Val Val Val
 290 295 300
 Gly Ala Pro Asn Ile Gln Asp Phe Ala Pro Ser Pro Gly Ser Ile Leu
 305 310 315 320
 His Ile Lys Glu Ile Glu Asp Val Glu Ser Val Ala Lys Thr Met Arg
 325 330 335
 Tyr Leu Ala Glu Asn Pro Glu Ala Tyr Asn Gln Ser Leu Arg Trp Lys
 340 345 350
 Tyr Glu Gly Pro Ser Asp Ser Phe Lys Ala Leu Val Asp Met Ala Ala
 355 360 365
 Val His Ser Ser Cys Arg Leu Cys Ile His Leu Ala Thr Val Ser Arg
 370 375 380
 Glu Lys Glu Glu Asn Asn Pro Ser Leu Lys Arg Arg Pro Cys Lys Cys
 385 390 395 400
 Thr Arg Gly Pro Glu Thr Val Tyr His Ile Tyr Val Arg Glu Arg Gly
 405 410 415
 Arg Phe Glu Met Glu Ser Ile Tyr Leu Arg Ser Ser Asn Leu Thr Leu
 420 425 430
 Asn Ala Val Lys Ala Ala Val Val Leu Lys Phe Thr Ser Leu Asn Leu
 435 440 445
 Val Pro Val Trp Lys Thr Glu Arg Pro Glu Val Ile Arg Gly Gly Ser
 450 455 460
 Ala Leu Lys Leu Tyr Lys Ile Tyr Pro Ile Gly Leu Thr Gln Arg Gln
 465 470 475 480
 Ala Leu Tyr Thr Phe Ser Phe Lys Gly Asp Ala Asp Phe Arg Ser His
 485 490 495
 Leu Glu Asn Asn Pro Cys Ala Lys Phe Glu Val Ile Phe Val
 500 505 510

FIG.6 b

9/16

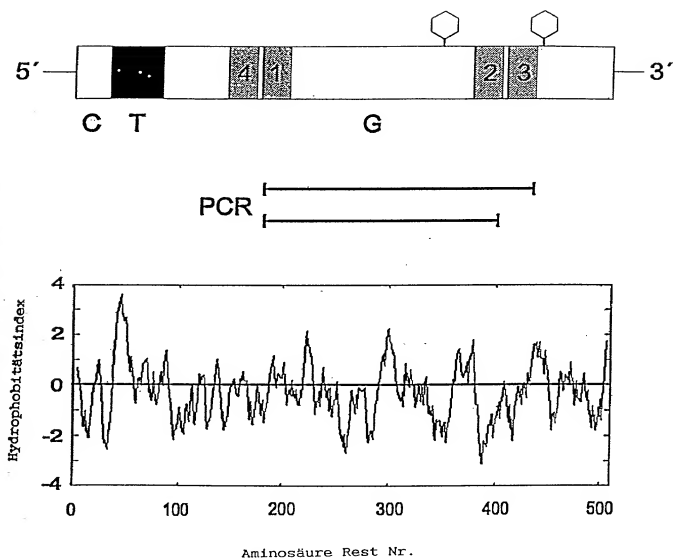


FIG.7

10/16

Transferase Art konserviertes Motiv

FucT-C3	Mungo Bohne	# ^a	267-EALKHKYFSLAFENSNEEDYVTEKFFQ-SIVAGTVP
FucT-III	Mensch	P21217	236-ETLSRYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-III	Schimpanse	O19058	247-ETLSRYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-V	Mensch	Q11128	249-ETLSRYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-V	Schimpanse	P56433	249-ETLSRYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-VI	Mensch	P51993	235-ETLSRYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-VI	Schimpanse	P56434	235-ETLSRYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-III	Rind	Q11126	240-KQLSQYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-?	chin. Hamster	O35886	237-GTLARYKFTYLAFENSLHPDYITEKLMWNALEAWVP
FucT-VII	Mensch	Q11130	217-PTVAQYRFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-VII	Maus	Q11131	264-PTLARYRFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-VII	<i>Schistosoma mansoni</i>	O76204	226-PTLARYRFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-IV	Mensch	P22083	277-HTVARYKFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-IV	Maus	Q11127	305-HTVARYKFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-IV	Ratte	Q62994	305-HTVARYKFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-IV	Huhn	Q98952	228-KTVSAKFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT-IX	Maus	O88819	233-PTISTCKFTYLAFENSQHRDYITEKLMWNALEAWVP
FucT	<i>Dicynostium discoidum</i>	O76544	247-DVLRKRYNFTYLAFENSQHRDYITEKLMWNALEAWVP
hpFucT1	<i>Helicobacter pylori</i>	O32631	106-EFLSQYKFNLCFENSQGYGVTEKIID-AYFSHTIP
hpFucT2	<i>Helicobacter pylori</i>	O30511	227-EFLSQYKFNLCFENSQGYGVTEKIID-AYFSHTIP
CEFT-1	<i>Caenorhabditis elegans</i>	Q21362	303-MLDIDYHFYVTFENSICEDYVTEKLMWNALEAWVP

^a Zugriffsnummer für die SwissProt Protein Sequenz Datenbank

FIG.8

11/16

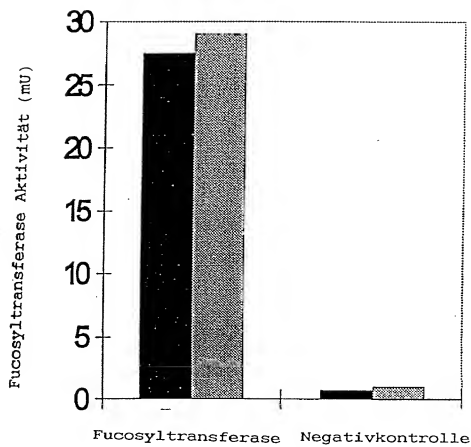


FIG.9

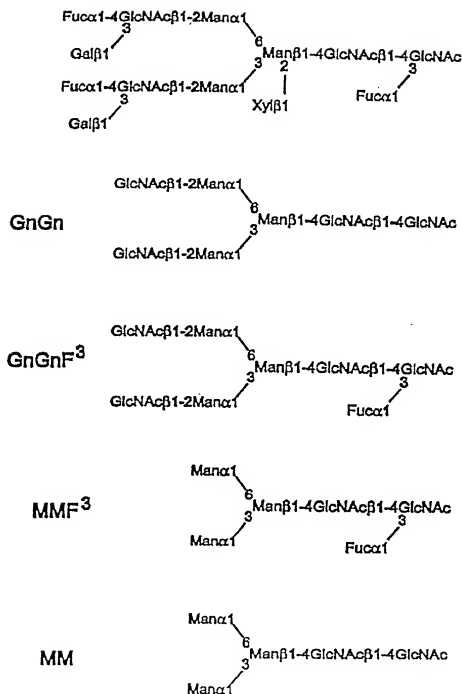


FIG.10 a

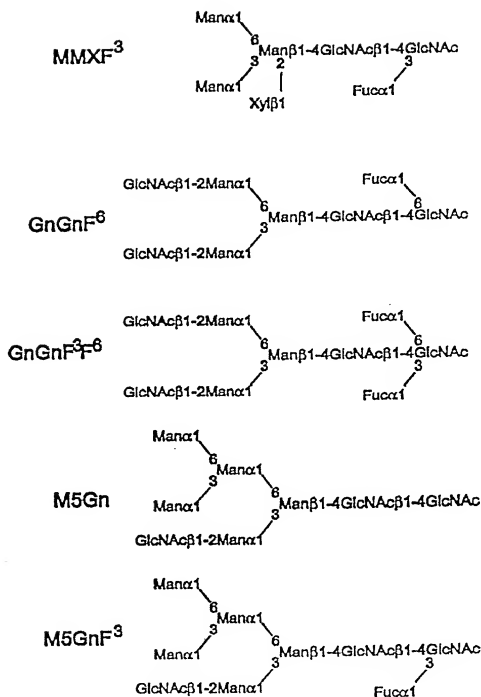


FIG.10 b

14/16

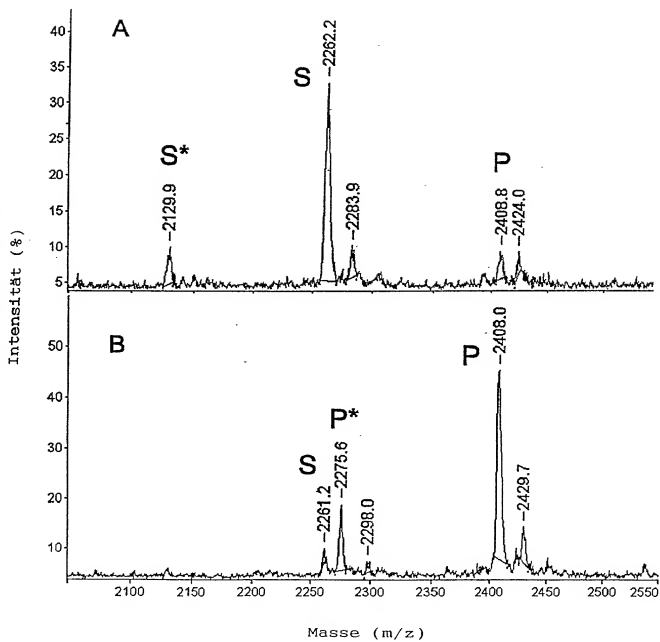
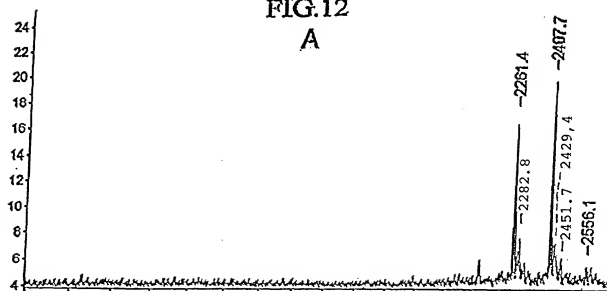


FIG.11

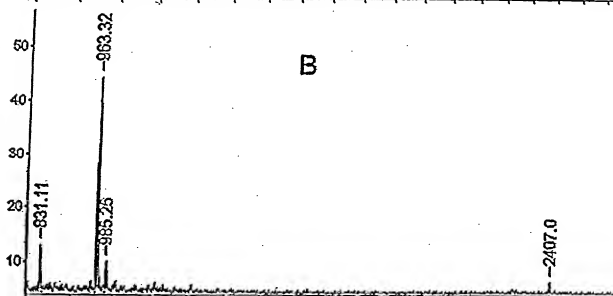
15/16

FIG.12

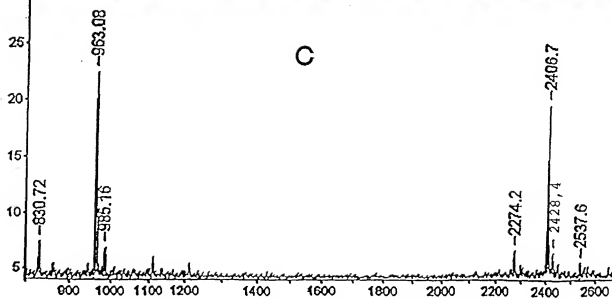
A



B



C

Masse (m/z)

16/16

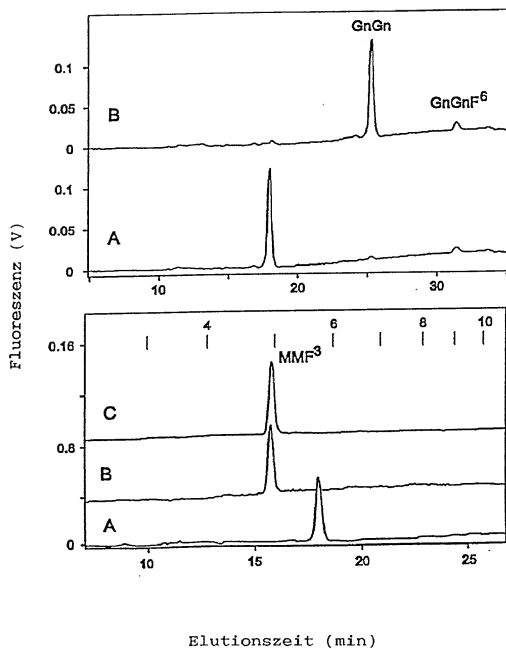


FIG.13

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to Provisional and PCT International Applications)

ATTORNEY'S DOCKET NUMBER

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Fucosyl Transferase Gene

the specification of which (check only one item below):

- ☐ is attached hereto.
☐ was filed as United States application
Number _____
on _____
and was amended
on _____ (if applicable).

- ☒ was filed as PCT international application
Number PCT/AT00/00040
on 17 February 2000
and was amended under PCT Article 19
on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 35, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Austria	A 270/99	18 February 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED)
(Includes Reference to Provisional and PCT International Applications)

ATTORNEY'S DOCKET NO.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS

STATUS (check one)

U.S. APPLICATION NUMBER

U.S. FILING DATE

PATENTED

PENDING

ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.

PCT APPLICATION NO.

PCT FILING DATE

U.S. APPLICATION NUMBERS
ASSIGNED (if any)

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	<u>47,337</u>	Samuel C. Miller, III	<u>27,360</u>	Robert M. Schulman	<u>31,196</u>
Peter H. Smolka	<u>15,913</u>	Ralph L. Fredland, Jr.	<u>16,110</u>	William C. Rowland	<u>30,888</u>
Robert S. Swecker	<u>19,885</u>	Robert G. Mukai	<u>28,531</u>	T. Gene Dillashanty	<u>25,423</u>
Blair N. Mandros	<u>22,124</u>	George A. Hovanec, Jr.	<u>28,223</u>	Patrick C. Keane	<u>32,858</u>
Benton S. Duffett, Jr.	<u>22,030</u>	James A. LaBarre	<u>28,632</u>	Bruce J. Boggs, Jr.	<u>37,344</u>
Joseph R. Magnone	<u>24,239</u>	E. Joseph Gess	<u>28,510</u>	William H. Benz	<u>25,952</u>
Norman H. Stepano	<u>22,716</u>	R. Danny Huntington	<u>27,903</u>	Peter K. Skiff	<u>31,917</u>
Ronald L. Grudzicki	<u>24,370</u>	Eric H. Weisblatt	<u>30,505</u>	Richard J. McGrath	<u>29,199</u>
Frederick G. Michaud, Jr.	<u>26,003</u>	James W. Peterson	<u>26,057</u>	Matthew L. Schneider	<u>32,814</u>
Alan E. Kopecki	<u>25,813</u>	Teresa Stanek Rea	<u>30,427</u>	Michael G. Savage	<u>32,496</u>
Regis E. Slutter	<u>26,999</u>	Robert E. Krebs	<u>25,885</u>	Gerald F. Swiss	<u>30,113</u>

and: _____

Address all correspondence to: **BURNS, DOANE, SWECKER & MATHIS, L.L.P.**
P.O. Box 1404
Alexandria, Virginia 22313-1404

Address all telephone calls to: _____ at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)		ATTORNEY'S DOCKET NO.
FULL NAME OF SOLE OR FIRST INVENTOR Friedrich ALTMANN	SIGNATURE <i>[Signature]</i>	DATE 7 Aug 2001
RESIDENCE Muthgasse 18, A-1190 Wien, Austria	CITIZENSHIP Austria	
POST OFFICE ADDRESS Institut für Chemie, Universität für Bodenkultur, Muthgasse 18, A-1190 Wien, Austria		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		

United States Patent & Trademark Office
Office of Initial Patent Examination – Scanning Division



Application deficiencies found during scanning:

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ *Scanned copy is best available.*

*Drawings figure 2 and 3
are dark*